EB509797152US

10/613639

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re:	US Patent No. 7,214,364
Issued:	8 May 2007
Inventor:	Alan Bruce Montgomery; Manfred Keller, Frank-Christophe Lintz
Assignee:	Gilead Sciences Inc. (formerly Corus Pharma, Inc.)
For:	INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS
Serial No.:	10/613,639
Filed:	3 July 2003

Commissioner of Patents
Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

Re: Request for Patent Term Extension Under 35 U.S.C. §156 for U.S. Patent No. 7,214,364

Transmitted herewith are the application papers of Gilead Sciences, Inc., dated **31 March 2010** for extension of the term of U.S. Patent No. 7,214,364 under 35 U.S.C. §156, based on the regulatory review period of CAYSTON® (Aztreonam for Inhalation Solution), together with two duplicate copies as required under 37 C.F.R. §1.740(b) for a total of two copies, one original and a return receipt postcard, as requested by Ms. Mary Till of the Office of Patent Legal Administration.

As set forth in 37 C.F.R.. §1.20(j), please charge the sum of $1,120.00 to Deposit Account No. 07-1250 for the filing of this application for extension of patent term. Also, please charge any underpayment, or any additional fees that may be required, or credit any overpayment, to Deposit Account No. 07-1250. Two copies of this paper are enclosed.

Respectfully submitted,
Gilead Sciences, Inc.

Dated: 31 March 2010

Lorie Ann Morgan
Attorney for Applicant
Reg. No. 38,181

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Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Commissioner of Patents
Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

Re: Application for Patent Term Extension of U.S. Patent No. 7,214,364 Under 35 U.S.C. §156 for CAYSTON® (Aztreonam for Inhalation Solution)

Applicant, Gilead Sciences, Inc., a corporation of the State of California, having a place of business at 333 Lakeside Drive, Foster City California, 94404, United States of America, represents that it is the owner of the entire right, title and interest in and to Letters Patent of the United States No. 7,214,364 granted to Alan Bruce Montgomery on 8 May 2007, for INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS, by virtue of assignments recorded in the United States Patent and Trademark Office on November 10, 2003 at Reel 014674, Frame 0290; on October 25, 2004 at Reel 015290, Frame 0229; and on October 22, 2008 at Reel 021719, Frame 0001. A petition to correct inventorship under 37 C.F.R. §1.324 was filed September 16, 2009, along with a request for certificate of correction. To date the Office has not yet issued a decision on the petition to correct inventorship. Copies of the Assignments and Notices of Recordation are enclosed as **EXHIBIT 1**.

Submitted herewith is the Notice of Acceptance of the Power of Attorney filed 4 March 2010 on behalf of Gilead Sciences, Inc. which establishes the right of Gilead Sciences, Inc. as assignee, to take action in the Patent and Trademark Office in connection with this patent, and grants power of attorney to the named registered patent attorneys.

Applicant further represents that Applicant is the holder of the regulatory approval granted by the Food and Drug Administration ("FDA") for CAYSTON® (Aztreonam for Inhalation Solution). A copy of the FDA Approval Letter for CAYSTON® is attached hereto as **EXHIBIT 2**.

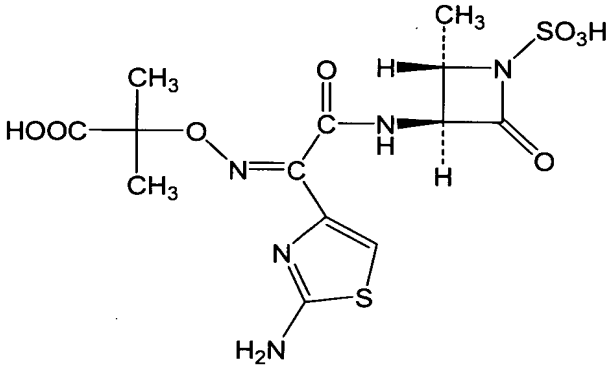
Pursuant to 37 C.F.R. 1.730, Applicant hereby applies for an extension of the term of U.S. Patent No. 7,214,364 under 35 U.S.C. §156 of **794 days**, based on the materials set forth herein and the accompanying papers.

For convenience, the numbered paragraphs (1) through (15) herein correspond to paragraphs (1) through (15) of 37 C.F.R. 1.740(a).

(1) Applicant's approved product is CAYSTON® (Aztreonam for Inhalation Solution). The only active ingredient in CAYSTON® is aztreonam formulated with lysine specifically for inhalation. Identification of the approved product is provided as follows:

Chemical Name: (*Z*)-2-[[[(2-amino-4-thiazolyl)[[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl]methylene]amino]oxy]-2-methylpropionic acid.

Structural formula:



Physical Description: CAYSTON® is a white to off-white powder. CAYSTON® is sterile, hygroscopic, and light sensitive. Once reconstituted with the supplied diluent, the pH range is 4.5 to 6.0.

A copy of the package insert (product label) approved by the FDA as part of New Drug Application 50-814 (NDA) is attached hereto as **EXHIBIT 3**.

(2) CAYSTON® (Aztreonam for Inhalation Solution) was subject to regulatory review under the Federal Food, Drug and Cosmetic Act, section 505(b) which is codified at 21 U.S.C. §355(b).

(3) CAYSTON® (Aztreonam for Inhalation Solution) received permission for commercial marketing and use under section 505(b) of the Federal Food, Drug and Cosmetic Act, section 505(b) (21 U.S.C. §355(b)) on 22 February 2010. See **EXHIBIT 2**. CAYSTON® is indicated to improve respiratory symptoms in cystic fibrosis (CF) patients with *Pseudomonas aeruginosa*.

(4) Aztreonam formulated with lysine for inhalation, has not previously been approved for commercial marketing or use under the Federal Food Drug and

Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act. Aztreonam formulated with arginine for parenteral administration was previously approved for commercial marketing or use under the Federal Food Drug and Cosmetic Act in 1986, under the trade name AZACTAM®. The AZACTAM® product Label is provided at **EXHIBIT 4**. A more detailed explanation of these circumstances is provided at item (13) below.

(5) This application for extension of patent term under 35 U.S.C. 156 is being submitted within the permitted 60-day period, which will expire on 22 April 2010.

(6) The complete identification of the patent for which extension of term is being sought is as follows:

U.S. Patent No.:	7,214,364
For:	INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS
Inventor:	Alan Bruce Montgomery; Manfred Keller; Frank-Christophe Lintz
Assignee:	Gilead Sciences, Inc.
Issued:	8 May 2007
Expiration Date:	20 December 2021

(7) A complete copy of the patent identified in paragraph (6) above is attached hereto as **EXHIBIT 5**.

(8) A copy of the Terminal Disclaimers filed for U.S. Patent 7,214,364, disclaiming the portion of patent term extending beyond the expiration date of U.S. Application Serial No. 10/654,815 (now U.S. Patent No. 7,208,141); and U.S. Application Serial No. 10/882,985 (now U.S. Patent No. 7,138,419) are attached hereto as **EXHIBIT 6**.

Copies of the certificates of correction that have issued for this patent at attached as **EXHIBIT 7**.

Maintenance fees have not yet come due for U.S. Patent 7,214,364. A copy of the Patent Maintenance Fee Statement providing the next window for payment and stating that currently there are no fees due is attached hereto as **EXHIBIT 8**.

No reexamination certificate or reissue patent exists in respect of U.S. Patent 7,214,364.

(9) U.S. Patent 7,214,364 claims the approved pharmaceutical composition including the approved product. Pursuant to 37 CFR §1.740(9), Applicant herein below lists each applicable patent claim and demonstrates the manner in

which at least one applicable claim reads on the approved product or method of using the approved product.

(a) Claim 1 reads as follows:
"An inhalable composition comprising aztreonam lysinate, said composition suitable for the treatment of pulmonary bacterial infections caused by gram-negative bacteria, wherein said aztreonam lysinate is prepared as an inhalable dry powder having a particle size with a mass medium average diameter from about 1 to about 5 μm.

Claim 1 reads on the approved product, CAYSTON® (Aztreonam for Inhalation Solution), because the approved product contains lyophilized aztreonam (75 mg) and lysine (46.7 mg) which is reconstituted with 1 mL sterile diluent (0.17% sodium chloride) for administration by inhalation using the Altera® Nebulizer System, and is indicated to improve respiratory symptoms in cystic fibrosis (CF) patients with *Pseudomonas aeruginosa*, a gram-negative bacterial infection. The Altera® nebulizer system produces aerosol particles having a mass median aerodynamic diameter from about 1 to about 5 μm.

(b) Claim 2 reads as follows:
"The composition of claim 1 wherein the aztreonam lysinate is alpha aztreonam lysinate."

(c) Claim 6 reads as follows:
"The composition of claim 1 wherein the gram-negative bacteria is a multidrug resistant *Pseudomonas aeruginosa*."

(10) The relevant dates and information pursuant to 35 U.S.C 156(g) necessary to enable the Secretary of Health and Human Resources to determine the applicable regulatory review period are as follows:

(a) Effective Date and Number of the IND
The Investigational New Drug Application ("IND") for CAYSTON® (Aztreonam for Inhalation Solution) was filed 14 April 2003 and became effective 14 May 2003; it was designated IND No. 64,402 (Safety and Tolerability Study of Ascending Single Doses of Aztreonam for Inhalation (AI) in Patients with cystic Fibrosis).

(b) Issue Date of Patent
US Patent No. 7,214,364 issued 8 May 2007 and claims a new drug product. See **EXHIBIT 5**.

(c) Submission Date and Number of NDA
The NDA for CAYSTON® (Aztreonam for Inhalation Solution) was submitted on 16 November 2007 and was designated NDA No. 50-814.

(d) Approval Date of NDA
NDA No. 50-814 for CAYSTON® (Aztreonam for Inhalation Solution) was approved by the FDA on 22 February 2010. See **EXHIBIT 2**.

(11) A brief description of the significant activities undertaken by Applicant during both the IND and NDA regulatory periods and the dates on which such activities took place, is presented in a chronological form and is attached hereto as **EXHIBIT 9**, "Due Diligence Log". Applicant reserves the right to supplement the chronology of **EXHIBIT 9** with materials from which it was derived or other evidence related to Applicant's conduct in obtaining the approval of CAYSTON® (Aztreonam for Inhalation Solution) *See, e.g.*, 21 CFR §60.32.

(12) Applicant is of the opinion that U.S. Patent 7,214,364 is eligible for a **794-day** extension, subject to the 14-year limitation under 35 U.S.C. 156(c)(3).

(a) Applicant has satisfied the eligibility criteria necessary to obtain a patent term extension pursuant to 35 U.S.C. 156 as follows:

35 U.S.C. 156(a):
U.S. Patent No. 7,214,364 claims the approved product and a method of using the approved product.

35 U.S.C. 156(a)(1)
The term of U.S. Patent No. 7,214,364 has not yet expired before submission of this application under 35 U.S.C. 156(d)(1).

35 U.S.C. 156(a)(2)
The term of U.S. Patent No. 7,214,364 has never been extended under 35 U.S.C. 156(e)(1).

35 U.S.C. 156(a)(3)
The application for extension is submitted by the owner of record in accordance with the requirements of 35 U.S.C. 156(d) and 37 CFR 1.730.

35 U.S.C. 156(a)(4)
The approved product, CAYSTON® (Aztreonam for Inhalation Solution), has been subject to a regulatory review period before its commercial marketing or use.

35 U.S.C. 156(a)(5)(A)
The commercial marketing or use of the approved product, CAYSTON® (Aztreonam for Inhalation Solution), after the regulatory review period is the first permitted commercial marketing or use of the approved product under the provisions under which such regulatory review period occurred.

35 U.S.C. 156(c)(4)
No patent has to this date been extended under subsection 35 U.S.C. 156(e)(1), for the regulatory review period which forms the basis for this application for extension of the term of Patent No. 7,214,364.

Pursuant to 37 CFR §1.785(b), Applicant is concurrently filing multiple applications for extension, which seek the extension of patent term of three (3) patents (i.e., U.S. Patent No. 7,208,141, 7,214,364, and 7,427,633) based upon the same regulatory review period, and expressly requests the opportunity to elect a particular patent for extension once the office confirms that all patents are eligible for extension pursuant to 37 CFR §1.710.

(b) Applicant herewith claims a patent term extension of **794 days**, as limited by the 14-year limitation under 35 U.S.C. 156(c)(3), for U.S. Patent No. 7,214,364 pursuant to U.S.C. 156(g) as follows:

(1) Pursuant to 37 CFR §1.775(b), the length of extension is equal to the regulatory review period for the approved product, reduced as appropriate pursuant to paragraphs (d)(1) through (d)(6) of 37 CFR §1.775.

(2) Pursuant to 37 CFR §1.775(c), the regulatory review period is the sum of: (i) the number of days in the period beginning on the date the exemption under subsection 505 of the Federal Food, Drug and Cosmetic (FFDCA) became effective for the approved product and ending on the date the NDA was initially submitted under subsection 505 of the FFDCA; and (ii) the number of days in the period beginning on the date the NDA was initially submitted under subsection 505 of the FFDCA and ending on the date the NDA was approved.

The Investigational New Drug Application ("IND") for CAYSTON® (Aztreonam for Inhalation Solution) was filed 14 April 2003 and became effective 14 May 2003. The NDA for CAYSTON® (Aztreonam for Inhalation Solution) was submitted on 16 November 2007 and approved on 22 Feb 2010. Thus, the regulatory review period is the sum of the period from 14 May 2003 to 16 November 2007 and the period from 16 November 2007 to 22 Feb 2010. This sum equals:
1647 days + 830 days = 2477 days.

(3) Pursuant to 37 CFR §1.775(d)(1)(i), the number of days in the regulatory review period which were on or before the date on which the patent issued must be subtracted.

US Patent No. 7,214,364 issued 8 May 2007. The number of days in the review period which were before the patent issued is the period from 14 May 2003 to 8 May 2007 which equals 1455 days. The regulatory review period is therefore reduced to the period beginning on 8 May 2007 to 16 November 2007 and from 16 November 2007 to 22 Feb 2010. The sum of these periods is:
192 days + 830 days = 1022 days

(4) 37 CFR §1.775(d)(1)(ii) is not applicable.

(5) Pursuant to 37 CFR §1.775(d)(1)(iii), the regulatory review period must then be reduced by one-half of the days remaining in the period defined in 37 CFR §1.775(c)(1). This is one-half of period from 8 May 2007 to 16 November 2007 or one-half of 192 days, or 96 days. The reduced regulatory review period after subtraction is therefore 96 days + 830 days = **926 days**.

(6) Pursuant to 37 CFR §1.775(d)(2), the reduced regulatory review period of 926 days must be added to the expiration date of U.S. Patent No. 7,214,364. The expiration of U.S. Patent 7,214,364, by virtue of the terminal disclaimers, is 20 December 2021. Adding 926 days to 20 December 2021 gives an extended expiry date of **3 July 2024**.

(7) Pursuant to 37 CFR §1.775(d)(3), adding 14 years to the date of approval of the application under section 505 of the FFDCA gives: 22 Feb 2010 + 14 years = **22 February 2024**.

(8) Pursuant to 37 CFR §1.775(d)(4), comparing the dates for the ends of the periods obtained pursuant to paragraphs (d)(2) and (d)(3) and selecting the earlier date results in an extended expiry date of **22 February 2024**. The expiration date of U.S. Patent 7,214,364 is therefore limited by the provisions of 35 U.S.C. 156(c)(3).

(9) Pursuant to 37 CFR §1.775(d)(5)(i), if the original patent was issued after 24 September 1984, (i) by adding 5 years to the original expiration date of the patent or any earlier date set by terminal disclaimer results in: 20 December 2021 + 5 years = 20 December 2026.

(10) Pursuant to 37 CFR §1.775(d)(5)(ii) the dates obtained pursuant to paragraphs (d)(4) and (d)(5)(i) are compared and the earlier date selected. Selecting the earlier date of 22 February 2024 and 20 December 2026 results in an extended expiry date of **22 February 2024.** The expiration date of U.S. Patent 7,214,364 is therefore not limited by the provisions of 35 U.S.C. 156(g)(6).

(11) 37 CFR §1.775(d)(6) is not applicable.

(c) Applicant hereby claims an extended expiry date of **22 February 2024** for U.S. Patent 7,214,364 pursuant to 35 U.S.C. 156(c)(3).

(13) The Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to any determinations to be made relative to the application for extension. The following information is provided for consideration.

(a) Applicant respectfully submits that the product covered by U.S. Patent No. 7,214,364 is the "first permitted commercial marketing or use of the product" under 35 U.S.C. §156(a)(5)(A) (2006). Section 156(f)(1)(A) defines the term "product" as "a drug product" which in turn, is defined as "the active ingredient of ... a new drug, antibiotic drug, or human biological product... including any salt or ester of the active ingredient, as a single entity or in combination with another active ingredient." 35 U.S.C. §156(f)(2)(2006).

(b) Applicant's NDA, approval letter (**EXHIBIT 1**), and approved product label (**EXHIBIT 3**) all identify the product as "CAYSTON® (Aztreonam for Inhalation Solution)" and further state that "CAYSTON is not for intravenous or intramuscular administration." The "Description" in the approved product label states:

> "A dose of CAYSTON consists of a 2 mL amber glass vial containing lyophilized aztreonam (75 mg) and lysine (46.7 mg), and a low-density polyethylene ampoule containing 1 mL sterile diluent (0.17% sodium chloride). The reconstituted solution is for inhalation. The formulation contains no preservatives or arginine." *See*, CAYSTON® Approved Product Label, page 7 - **EXHIBIT 3** (emphasis added).

The CAYSTON® product therefore contains aztreonam lysine (i.e., aztreonam formulated with lysine) for inhalation. Thus, the approved product is described as containing lysine, expressly designated "for inhalation" and expressly excludes arginine.

(c) The approved product label also states: "The active ingredient in CAYSTON® is aztreonam," and further states "Initial U.S. Approval: 1986" for the active ingredient. *See*, CAYSTON® Approved Product Label, page 7 and 1, respectively. In 1986, Bristol-Myers Squibb Company received approval to market a product identified as "AZACTAM® (aztreonam for injection, USP)". Applicant's NDA relied in part, on data relating to aztreonam that was submitted to the FDA in the application for approval to market AZACTAM® (aztreonam for injection, USP). AZACTAM® (aztreonam for injection, USP) contains aztreonam arginine (i.e., aztreonam formulated with arginine). The AZACTAM® product label expressly states: "the product is for intramuscular or intravenous use." *See*, AZACTAM® Label, page 1. AZACTAM® was never approved for administration by inhalation and is believed to be unsuitable for inhalation due to the presence of arginine. Hans-Joachim Dietzsch, et al., Cystic Fibrosis: Comparison of Two Mucolytic Drugs for Inhalation Treatment (Acetylcysteine and Arginine Hydrochloride), *Pediatrics* (1975) 55(1):96-100, demonstrates that arginine may cause

airway inflammation after chronic inhaled administration to cystic fibrosis patients. See, **EXHIBIT 10**. The conclusions of the Dietzsch study were not overcome by the report of F.J. Dapena et al., "Inhaled Aztreonam Therapy in Patients with Cystic Fibrosis Colonized with *Pseudomonas aeruginosa*" *Anals Espanoles de Pediatria* (1994) 40(3) (see, **EXHIBIT 11**) wherein AZACTAM® (500 mg or 1 g) was administered twice per day by inhalation to 19 cystic fibrosis patients following pre-treatment with normal saline alone or in combination with a bronchodilator.

(d) Applicant invented CAYSTON® (Aztreonam for Inhalation Solution), containing aztreonam lysine, specifically for administration by inhalation in cystic fibrosis patients. CAYSTON® qualified as a new drug under §201(p) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. §321(p) (2006) and accordingly, it required approval by the FDA before it could be commercially marketed and sold. Applicant filed IND No. 64,402 to evaluate the safety and tolerability of ascending single doses of Aztreonam for Inhalation Solution (AI) in patients with cystic fibrosis. Applicant sought and obtained approval for NDA No. 50-814 to market CAYSTON® (Aztreonam for Inhalation Solution) for the improvement of respiratory symptoms in cystic fibrosis (CF) patients with *Pseudomonas aeruginosa*. Applicant's NDA was approved 22 February 2010. The product, as identified on the FDA approval letter and approved product label is "CAYSTON® (Aztreonam for Inhalation Solution)."

(e) Applicant believes that granting a patent term extension for CAYSTON® (Aztreonam for Inhalation Solution) is consistent with the decision of the Federal Circuit Court of the Eastern District of Virginia in *Photocure ASA v. Dudas et al.*, 622 F. Supp.2d 338 (E.D.Va. Mar 2009) (on appeal), and the decision of the Court of Appeals for the Federal Circuit in *Glaxo Operations UK Ltd. V. Quigg*, 894 F.2d 392 (Fed. Cir. 1990). In *Photocure*, the court overturned the U.S. PTO's denial of a patent term extension under 35 U.S.C. §156, for Metvixia™, based on the prior approval of Levulan™. The active ingredient of Levulan™ is aminolevulinic acid HCl whereas the active ingredient of Metvixia™ is methyl aminoevulinate hydrochloride (MAL HCl). According to the U.S. PTO, both drugs shared the common active moiety, aminolevulinic acid (ALA), and therefore they contain the same "product" under section 156(f). As a consequence, the Office determined that the prior FDA approval of Levulan™ rendered Metvixia™ ineligible for patent term extension under 35 U.S.C. §156, on the grounds that the FDA approval upon which the request for patent term extension was based was not the first approval of "the product". The court reversed the U.S. PTO's decision to apply the active moiety interpretation and deny the patent term extension under §156(a)(5)(A) and held that such interpretation was contrary to the plain meaning of the statute and thus did not constitute a reasonable interpretation of the term "the product" in the statute. The court found that the active ingredient of Metvixia™ was MAL HCl and not ALA because MAL HCl is the ingredient physically present in Metvixia™ that permits the drug to work effectively and ALA does not exist in Metvixia™.

Like Metvixia™, the instant product qualified as a new drug under §201(p) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. §321(p) (2006) and accordingly, it required approval by the FDA before it could be commercially marketed and sold. Under the holding of *Photocure*, Applicant respectfully submits that the "active ingredient" in CAYSTON® is "aztreonam lysine," since that is the critical compound in CAYSTON® that enables the drug to be administered by inhalation for the approved indication, i.e., improvement of respiratory symptoms in CF patients with *Pseudomonas aeruginosa*. CAYSTON® does not include aztreonam arginine and aztreonam lysine is neither the same as aztreonam arginine nor a salt or ester of aztreonam arginine. Therefore, CAYSTON® is the first approved marketing or use of a drug containing the product "aztreonam lysine" or "Aztreonam for Inhalation".

(f) In *Glaxo Operations UK Ltd. V. Quigg*, 894 F.2d 392 (Fed. Cir. 1990), the Federal Circuit Court similarly ruled that the ester form of an active moiety was eligible for a patent term extension even though salt forms of the same active moiety had previously been approved. The Court identified the "active ingredient" in CEFTIN® as the approved ester form, cefuroxime axetil, rather than cefuroxime, and found that the FDA had not previously approved any salt or ester forms of cefuroxime axetil, even though it had approved salt forms of cefuroxime.

(g) Applicant respectfully submits that *Pfizer, Inc. v. Dr. Reddy's Labs.*, Ltd., 395 F.3d 1361 (Fed. Cir. 2004) is inapposite. *Pfizer* did not address the question whether a later product was eligible for patent term extension under 35 U.S.C. §156. The question addressed in *Pfizer* was whether an innovator's patent was infringed during the patent term extension period by a different salt form of the same active ingredient. Further, it is respectfully submitted that the approved active ingredient of CAYSTON® is aztreonam lysine, which is neither a salt or ester of the approved active moiety of AZACTAM® (i.e., aztreonam arginine – **EXHIBIT 4**).

(h) In the present case, the previously approved product AZACTAM® (aztreonam for injection, USP) was formulated with arginine specifically for administration by injection. AZACTAM® is not approved for inhalation and is expressly defined "for injection." Furthermore, AZACTAM® contains arginine which renders it unsuitable for chronic administration by inhalation in cystic fibrosis patients. Accordingly, Applicant respectfully submits that under section 156(f), "the product" of AZACTAM® is "aztreonam arginine" or "Aztreonam for Injection" (as expressly stated in that product label – **EXHIBIT 3**).

(i) Applicant's product CAYSTON® (Aztreonam for Inhalation Solution) contains aztreonam lysine specifically for inhalation administration. Accordingly, Applicant respectfully submits that "the product" of CAYSTON® is "aztreonam lysine" or "Aztreonam for Inhalation Solution" (as expressly stated in the approved product label). Aztreonam lysine is the component of CAYSTON® which renders the product

effective for administration by inhalation. Furthermore, CAYSTON® does not contain arginine or aztreonam arginine. *See*, CAYSTON® Approved Product Label, page 7. Inasmuch as the FDA has never approved a salt or ester form of aztreonam lysine or aztreonam for inhalation solution, it is respectfully submitted that the instant approval is the first approval of the product under section 156 and accordingly U.S. Patent No. 7,214,364 is eligible for the requested patent term extension.

(14) The Commissioner of Patent and Trademarks is hereby authorized to charge deposit account number 07-1250 in the amount of $1120.00 for receiving and acting upon this application for extension of term. In the event the actual fees due in connection with Applicant's application for patent term extension differ from the amount specified above, the Commissioner is hereby authorized to credit any overpayment or charge any underpayment to Applicants' deposit account number 07-1250.

(15) Inquiries and correspondences relating to this application for patent term extension are to be directed to:

Frank P. Grassler
Vice President Intellectual Property
Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
(650) 522-1597

The undersigned hereby certifies that this Application for Extension of Patent Term Under 35 U.S.C. 156, including **EXHIBITS 1-11** and supporting papers, is being submitted together with two duplicate copies as required under 37 C.F.R. §1.740(b), for a total of two copies and one original, as requested by Ms. Mary Till of the Office of Patent Legal Administration.

Respectfully submitted,
Gilead Sciences, Inc.

By: _____
Lorie Ann Morgan
Attorney for Applicant
Reg. No. 38,181

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(650) 522-1597

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/613,639	07/03/2003	Alan Bruce Montgomery	S107

CONFIRMATION NO. 4624

25000
GILEAD SCIENCES INC
333 LAKESIDE DR
FOSTER CITY, CA 94404

POA ACCEPTANCE LETTER

Date Mailed: 03/12/2010

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 03/04/2010.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/hsarwari/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

EXHIBIT 1

UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

JUNE 01, 2004

PETERS, VERNY, JONES & SCHMITT

102599150A

PETERS, VERNY, JONES & SCHMITT LLP
HANA VERNY
385 SHERMAN AVENUE, SUITE 6
PALO ALTO, CA 94306

PTAS

JUN 0 9 2004

RECEIVED

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

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PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 11/10/2003

REEL/FRAME: 014674/0290
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).
DOCKET NUMBER: 3818.02-5

ASSIGNOR:
MONTGOMERY, ALAN BRUCE

DOC DATE: 07/03/2003

ASSIGNEE:
CORUS PHARMA, INC.
2025 FIRST AVENUE, SUITE 800
SEATTLE, WASHINGTON 98121

SERIAL NUMBER: 10613639
PATENT NUMBER:

FILING DATE: 07/03/2003
ISSUE DATE:

TITLE: INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS

MAURICE CARTER, PARALEGAL
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

FORM PTO-1595 (Modified)
(Rev. 03-01)
OMB No. 0651-0027 (exp.5/31/2002)
P08A/REV03

11-13-2003

Docket No.: 3818.02-5

SHEET

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

Tab settings ➔ ➔ ➔ ▼ ▼ ▼ ▼

102599150

To the Director of the United States Patent and Trademark Office: Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):
ALAN BRUCE MONTGOMERY

11.10-03

Additional names(s) of conveying party(ies) ☐ Yes ☒ No

2. Name and address of receiving party(ies):

Name: CORUS PHARMA, INC.

Address: 2025 FIRST AVENUE, SUITE 800

City: SEATTLE State/Prov.: WA

Country: UNITED STATES ZIP: 98121

Additional name(s) & address(es) ☐ Yes ☒ No

3. Nature of conveyance:

☒ Assignment ☐ Merger

☐ Security Agreement ☐ Change of Name

☐ Other

Execution Date: JULY 3, 2003

4. Application number(s) or patent numbers(s):

If this document is being filed together with a new application, the execution date of the application is:

Patent Application No.	Filing date	B. Patent No.(s)
10/613,639	JULY 3, 2003	

Additional numbers ☐ Yes ☒ No

OPR/FINANCE 03 NOV 10 AM 10:23

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: HANA VERNY

Registration No. 30,518

Address: PETERS, VERNY, JONES & SCHMITT LLP

385 SHERMAN AVENUE, SUITE 6

11/12/2003 DBYRNE 00000168 10613639
01 FC:8021 40.00 OP

City: PALO ALTO State/Prov.: CA

Country: UNITED STATES ZIP: 94306

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 3.41):.....$ 40.00

☒ Enclosed - Any excess or insufficiency should be credited or debited to deposit account

☐ Authorized to be charged to deposit account

8. Deposit account number:

16-1331

(Attach duplicate copy of this page if paying by deposit account)

11/12/2003 DBYRNE 00000168 10613639
02 FC:8021 40.00 OP

DO NOT USE THIS SPACE

9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

HANA VERNY — Name of Person Signing

Signature

NOVEMBER 7, 2003 — Date

4 — Total number of pages including cover sheet, attachments, and

Mail documents to be recorded with required cover sheet information to:
Mail Stop Assignment Recordation Services
Director of the United States Patent and Trademark Office

Assignment of Rights, Title and Interest in Invention (Multiple inventors; single assignee)	Docket No. 3818.02-5

This is an Assignment of the following rights, title and interest: (check all that apply):

- ☒ *United States of America rights, title and interest in the invention*
- ☒ *Foreign rights, title and interest in the invention*
- ☒ *United States Patent Application Serial No.* 10/027,113
  Date of Execution: _____ *Date of Filing:* DECEMBER 20, 2001
- ☒ *United States Provisional Patent Application Serial No.* 60/258,423
- ☐ *United States Patent No(s).* _____
- ☐ *International (PCT) Patent Application Serial No.* _____
- ☐ *Other (specify)* _____

Title of the Invention

INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS

Inventors (assignors)

Name	*Address*
ALAN BRUCE MONTGOMERY	3455 EVERGREEN PT. ROAD, MEDINA, WASHINGTON 98030

Assignee

Name	*Address*
CORUS PHARMA, INC.	2025 FIRST AVENUE, SUITE 800 SEATTLE, WA 98121

Assignment of Rights, Title and Interest in Invention (Multiple inventors; single assignee)	Docket No. 3818.02-5

Whereas, we, the above-identified Inventors, have invented certain new and useful improvements in the Invention identified above and described in the above-identified patent application(s) and/or patent(s) (hereinafter referred to as "Invention");

And, whereas we desire to assign our above-identified rights, title and interest in the Invention to the above-identified Assignee;

Now, this indenture witnesseth, that for good and valuable consideration, the receipt whereof is hereby acknowledged;

We hereby assign, sell and transfer our above-identified rights, title and interest in said Invention, said application(s) as identified above, including any divisions, continuations, and continuations-in-part thereof, and in and to any and all Letters Patent of the United States, and countries foreign thereto, which may be granted or have granted for said Invention, and in and to any and all reissues and reexaminations thereof, and in and to any and all priority rights, Convention rights, and other benefits accruing or to accrue to us with respect to the filing of applications for patents or securing of patents in the United States and countries foreign thereto, unto said Assignee;

And we hereby authorize and request the Director of the United States Patent and Trademark Office to issue any United States Letters Patent which may issue for said Invention to said Assignee, as assignee of the whole right, title and interest thereto;

And we further agree to sign and execute all necessary and lawful future documents, including applications for foreign patents, for filing divisions, continuations and continuations-in-part of said application for patent, and/or, for obtaining any reissue or reissues of any Letters Patent which may be granted for my aforesaid Invention, as the Assignee or its Designee(s) may from time to time require and prepare at its own expense.

Inventors' Signatures (if Notarization is desired, do not sign here and proceed to next page)

Name	*Signature/Date*
ALAN BRUCE MONTGOMERY	A C R Montgomery

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

700125764A

OCTOBER 27, 2004

PTAS

PETERS, VERNY, JONES & SCHMITT LLP
HANA VERNY
425 SHERMAN AVENUE, SUITE 230
PALO ALTO, CA 94306

UNITED STATES PATENT AND TRADEMARK OFFICE
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PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 10/25/2004 REEL/FRAME: 015290/0229
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).
DOCKET NUMBER: 3818.02-5

ASSIGNOR:
KELLER, MANFRED DOC DATE: 09/14/2004

ASSIGNOR:
LINTZ, FRANK-CHRISTOPHE DOC DATE: 09/10/2004

ASSIGNEE:
CORUS PHARMA, INC.
2025 FIRST AVENUE, SUITE 800
SEATTLE, WASHINGTON 98121

SERIAL NUMBER: 10613639 FILING DATE: 07/03/2003
PATENT NUMBER: ISSUE DATE:
TITLE: INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS

TARA WASHINGTON, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

10/25/2004 700125764

Doc...: 3818.02-5

FORM PTO-1595 (Modified)
(Rev. 03-01)
OMB No. 0651-0027 (exp.5/31/2002)
Patent and Trademark Office

RECORDATION FORM COVER SHEET
PATENTS ONLY

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Patent and Trademark Office

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To the Director of the United States Patent and Trademark Office: Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):
MANFRED KELLER
FRANK-CHRISTOPHE LINTZ

Additional name(s) of conveying party(ies) ☐ Yes ☒ No

2. Name and address of receiving party(ies):
Name: CORUS PHARMA, INC.
Address: 2025 FIRST AVENUE, SUITE 800

City: SEATTLE State/Prov.: WA
Country: UNITED STATES ZIP: 98121

Additional name(s) & address(es) ☐ Yes ☒ No

3. Nature of conveyance:
☒ Assignment ☐ Merger
☐ Security Agreement ☐ Change of Name
☐ Other

Execution Date: 09/14/2004; 09/10/2004

4. Application number(s) or patent numbers(s):
If this document is being filed together with a new application, the execution date of the application is:

Patent Application No.	Filing date	B. Patent No.(s)
10/613,639	JULY 3, 2003	

Additional numbers ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:
Name: HANA VERNY
Registration No. 30,518
Address: PETERS, VERNY, JONES & SCHMITT LLP
425 SHERMAN AVENUE, SUITE 230
City: PALO ALTO State/Prov.: CA
Country: UNITED STATES ZIP: 94306

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 3.41):.....$
☐ Enclosed - Any excess or insufficiency should be credited or debited to deposit account
☒ Authorized to be charged to deposit account

8. Deposit account number:
16-1331
(Attach duplicate copy of this page if paying by deposit account)

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9. Statement and signature.
To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

HANA VERNY
Name of Person Signing

Signature

OCTOBER 25, 2004
Date

Total number of pages including cover sheet, attachments, and
4

Mail documents to be recorded with required cover sheet information to:
Mail Stop Assignment Recordation Services
Director of the United States Patent and Trademark Office
P.O. Box 1450, Alexandria, VA 22313-1450

01 FC:481 40.00 OP

Assignment of Rights, Title and Interest in Invention (Multiple inventors; single assignee)	Docket No. 3818.02-5

This is an Assignment of the following rights, title and interest: (check all that apply):

- ☒ *United States of America rights, title and interest in the invention*
- ☒ *Foreign rights, title and interest in the invention*
- ☒ *United States Patent Application Serial No.* 10/613,639
  Date of Execution: _____ *Date of Filing:* JULY 3, 2003
- ☒ *United States Provisional Patent Application Serial No.* 60/258,423
- ☐ *United States Patent No(s).* _____
- ☐ *International (PCT) Patent Application Serial No.* _____
- ☐ *Other (specify)* _____

Title of the Invention

INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS

Inventors (assignors)

Name	*Address*
MANFRED KELLER	NEUNKIRCHNER STRASSE 60, 81379 MUNCHEN, GERMANY
FRANK-CHRISTOPHE LINTZ	WALDSPIELPLATZ 5, 82319 STARNBERG, GERMANY

Assignee

Name	*Address*
CORUS PHARMA, INC.	2025 FIRST AVENUE, SUITE 800 SEATTLE, WA 98121 UNITED STATES

AS03A/REV01

Assignment of Rights, Title and Interest in Invention (Multiple inventors; single assignee)	Docket No. 3818.02-5

Whereas, we, the above-identified Inventors, have invented certain new and useful improvements in the Invention identified above and described in the above-identified patent application(s) and/or patent(s) (hereinafter referred to as "Invention");

And, whereas we desire to assign our above-identified rights, title and interest in the Invention to the above-identified Assignee;

Now, this indenture witnesseth, that for good and valuable consideration, the receipt whereof is hereby acknowledged;

We hereby assign, sell and transfer our above-identified rights, title and interest in said Invention, said application(s) as identified above, including any divisions, continuations, and continuations-in-part thereof, and in and to any and all Letters Patent of the United States, and countries foreign thereto, which may be granted or have granted for said Invention, and in and to any and all reissues and reexaminations thereof, and in and to any and all priority rights, Convention rights, and other benefits accruing or to accrue to us with respect to the filing of applications for patents or securing of patents in the United States and countries foreign thereto, unto said Assignee;

And we hereby authorize and request the Director of the United States Patent and Trademark Office to issue any United States Letters Patent which may issue for said Invention to said Assignee, as assignee of the whole right, title and interest thereto;

And we further agree to sign and execute all necessary and lawful future documents, including applications for foreign patents, for filing divisions, continuations and continuations-in-part of said application for patent, and/or, for obtaining any reissue or reissues of any Letters Patent which may be granted for my aforesaid Invention, as the Assignee or its Designee(s) may from time to time require and prepare at its own expense.

Inventors' Signatures (if Notarization is desired, do not sign here and proceed to next page)

Name	*Signature/Date*
MANFRED KELLER	
FRANK-CHRISTOPHE LINTZ	Sep 16th 2004

Assignment of Rights, Title and Interest in Invention (Multiple inventors; single assignee)	Docket No. 3818.02-5

Whereas, we, the above-identified Inventors, have invented certain new and useful improvements in the Invention identified above and described in the above-identified patent application(s) and/or patent(s) (hereinafter referred to as "Invention");

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Inventors' Signatures (if Notarization is desired, do not sign here and proceed to next page)

Name	*Signature/Date*
MANFRED KELLER	Manfred Keller Sept. 14, 2004
FRANK-CHRISTOPHE LINTZ	

UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

500682693A

OCTOBER 23, 2008

PTAS

PETERS VERNY, LLP
425 SHERMAN AVENUE, SUITE 230
PALO ALTO, CA 94306

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RECORDATION DATE: 10/22/2008 REEL/FRAME: 021719/0001
NUMBER OF PAGES: 6

BRIEF: MERGER (SEE DOCUMENT FOR DETAILS).
DOCKET NUMBER: 3818.02-5 (HV)

ASSIGNOR:
CORUS PHARMA, INC. DOC DATE: 12/22/2006

ASSIGNEE:
GILEAD SCIENCES, INC.
333 LAKESIDE DRIVE
FOSTER CITY, CALIFORNIA 94404

SERIAL NUMBER: 10613639 FILING DATE: 07/03/2003
PATENT NUMBER: 7214364 ISSUE DATE: 05/08/2007
TITLE: INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS

KIMBERLY WHITE, EXAMINER
ASSIGNMENT SERVICES BRANCH
PUBLIC RECORDS DIVISION

PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

10/22/2008
500682693

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	MERGER
EFFECTIVE DATE:	12/22/2006

CONVEYING PARTY DATA

Name	Execution Date
CORUS PHARMA, INC.	12/22/2006

RECEIVING PARTY DATA

Name:	GILEAD SCIENCES, INC.
Street Address:	333 LAKESIDE DRIVE
City:	FOSTER CITY
State/Country:	CALIFORNIA
Postal Code:	94404

PROPERTY NUMBERS Total: 1

Property Type	Number
Patent Number:	7214364

CH $40.00 7214364

CORRESPONDENCE DATA

Fax Number: (650)324-1678
Correspondence will be sent via US Mail when the fax attempt is unsuccessful.
Phone: 6503241677
Email: melinda@petersverny.com
Correspondent Name: PETERS VERNY, LLP
Address Line 1: 425 SHERMAN AVENUE, SUITE 230
Address Line 4: PALO ALTO, CALIFORNIA 94306

ATTORNEY DOCKET NUMBER:	3818.02-5 (HV)
NAME OF SUBMITTER:	HANA VERNY (REG. NO. 30,518)

Total Attachments: 5
source=RECORDCVR#page1.tif
source=MERGERDOC#page1.tif
source=MERGERDOC#page2.tif

source=MERGERDOC#page3.tif
source=MERGERDOC#page4.tif

Delaware

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF OWNERSHIP, WHICH MERGES:

"CORUS PHARMA, INC.", A DELAWARE CORPORATION,

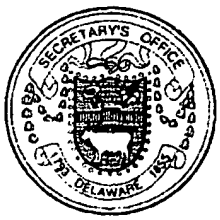
WITH AND INTO "GILEAD SCIENCES, INC." UNDER THE NAME OF "GILEAD SCIENCES, INC.", A CORPORATION ORGANIZED AND EXISTING UNDER THE LAWS OF THE STATE OF DELAWARE, AS RECEIVED AND FILED IN THIS OFFICE THE TWENTY-SECOND DAY OF DECEMBER, A.D. 2006, AT 9:44 O'CLOCK P.M.

AND I DO HEREBY FURTHER CERTIFY THAT THE EFFECTIVE DATE OF THE AFORESAID CERTIFICATE OF OWNERSHIP IS THE THIRTY-FIRST DAY OF DECEMBER, A.D. 2006, AT 11:59 O'CLOCK P.M.

A FILED COPY OF THIS CERTIFICATE HAS BEEN FORWARDED TO THE NEW CASTLE COUNTY RECORDER OF DEEDS.

2129876 8100M

061183751



Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

AUTHENTICATION: 5319206

DATE: 12-30-06

State of Delaware
Secretary of State
Division of Corporations
Delivered 09:44 PM 12/22/2006
FILED 09:44 PM 12/22/2006
SRV 061183751 - 2129876 FILE

CERTIFICATE OF OWNERSHIP AND MERGER
MERGING
CORUS PHARMA, INC.
WITH AND INTO
GILEAD SCIENCES, INC.

Pursuant to Section 253 of the
Delaware General Corporation Law

GILEAD SCIENCES, INC., a corporation organized and existing under the laws of the State of Delaware (this "Corporation"),

DOES HEREBY CERTIFY:

FIRST: That this Corporation was incorporated on June 22, 1987, pursuant to the Delaware General Corporation Law, the provisions of which permit the merger of a subsidiary corporation organized and existing under the laws of such State into a parent corporation organized and existing under the laws of such State.

SECOND: That this Corporation owns at least ninety percent (90%) of the outstanding shares of the common stock, $0.001 par value per share, of Corus Pharma, Inc., a corporation incorporated on January 2, 2001, pursuant to the Delaware General Corporation Law ("Corus"), and having no class of stock outstanding other than such common stock.

THIRD: That this Corporation, by the following resolutions of its Board of Directors, duly adopted at a meeting held on December 19, 2006, determined that, effective as of 11:59 p.m. EASTERN STANDARD TIME on December 31, 2006, Corus shall merge with and into the Corporation (the "Merger"), with the Corporation surviving the Merger:

MERGER

NOW, THEREFORE, BE IT RESOLVED, that the Board of Directors of Gilead Sciences, Inc. (the "Corporation") believes that the Merger is advisable and in the best interests of the Corporation, and the Board of Directors of the Corporation hereby approves the Merger and declares its advisability; and

FURTHER RESOLVED, that the officers of the Corporation be, and each of them hereby is, authorized, empowered and directed, in the name of and for and on behalf of the Corporation, to execute and deliver any agreements, certificates and other documents to consummate the Merger; and

FURTHER RESOLVED, that the officers of the Corporation be, and each of them hereby is, authorized and directed to take such further action as each may deem necessary or appropriate to carry out the intent of the above resolutions.

FOURTH: That the Merger has been approved by the holder of all of the outstanding stock of Corus entitled to vote thereon by written consent without a meeting in accordance with Section 228 of the Delaware General Corporation Law.

FIFTH: That the name of the surviving corporation is "Gilead Sciences, Inc."

SIXTH: That the Merger shall become effective at 11:59 p.m. EASTERN STANDARD TIME on December 31, 2006.

[Remainder of page intentionally left blank.]

IN WITNESS WHEREOF, Gilead Sciences, Inc. has caused this Certificate of Ownership and Merger to be executed in its corporate name as of the 22nd day of December, 2006.

GILEAD SCIENCES, INC.

By: _____
Name: John F. Milligan, Ph.D.
Title: Executive Vice President and Chief Financial Officer

ATTESTED TO:

By: _____
Name: Gregg H. Alton
Title: Senior Vice President and General Counsel of Gilead Sciences, Inc.

EXHIBIT 2

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

NDA 50-814

NDA APPROVAL

Gilead Sciences, Inc.
Attention: Jennifer Stephens
Director, Regulatory Affairs
2025 First Avenue, Suite PH
Seattle, Washington 98121

Dear Ms. Stephens:

Please refer to your new drug application (NDA) dated November 16, 2007, received November 16, 2007, submitted pursuant to section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act for Cayston (aztreonam for inhalation solution) in association with the Altera Nebulizer System which is the subject of 510(k) application K100380.

We acknowledge receipt of your submissions dated August 12, 21 and 26, September 11 and 22, October 15 and 30, and November 10 and 13, 2009, January 18 and 21, February 8, 9, 11 and 12, 2010.

The August 12, 2009, submission constituted a complete response to our September 16, 2008, action letter.

This new drug application provides for the use of Cayston (aztreonam for inhalation solution) to improve respiratory symptoms in cystic fibrosis (CF) patients with *Pseudomonas aeruginosa.*

We have completed our review of this application, as amended. It is approved, effective on the date of this letter, for use as recommended in the enclosed agreed-upon labeling text.

REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication(s) in pediatric patients unless this requirement is waived, deferred, or inapplicable.

Because this drug product for this indication has an orphan drug designation, you are exempt from this requirement.

POSTMARKETING REQUIREMENTS UNDER 505(o)

Section 505(o) of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute (section 505(o)(3)(A)).

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to assess the signal of serious risk of development of aztreonam resistance in *Pseudomonas aeruginosa* from cystic fibrosis (CF) patients.

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA has not yet been established and is not sufficient to assess this serious risk.

Therefore, based on appropriate scientific data, FDA has determined that you are required, to conduct the following:

1585-001 A prospective study in the United States which includes the five year period of time after introduction of Cayston (aztreonam for inhalation) to the market to determine if decreased susceptibility to aztreonam is increasing in *Pseudomonas aeruginosa* from cystic fibrosis (CF) patients. Provide a detailed protocol to the Agency for review and comment before commencing the study. Interim reports of changes in *P. aeruginosa* susceptibility from CF patients should be submitted annually for five years. After the first year, the report should be cumulative.

The information you submitted on January 18, 2010, states that you will conduct this study according to the following timetable:

Final Protocol Submission: 07/2010
First Interim Report: 01/2013, then annually
Study Completion Date: 04/2017
Final Report Submission: 01/2018

Submit the protocol to your IND 64,402, with a cross-reference letter to this NDA. Submit all interim and final reports to your NDA. Prominently identify the submission with the following wording in bold capital letters at the top of the first page of the submission, as appropriate:

- **REQUIRED POSTMARKETING PROTOCOL UNDER 505(o)**
- **REQUIRED POSTMARKETING FINAL REPORT UNDER 505(o)**
- **REQUIRED POSTMARKETING CORRESPONDENCE UNDER 505(o)**

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Section 506B of the FDCA, as well as 21 CFR 314.81(b)(2)(vii) requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 314.81(b)(2)(vii) to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii) provided that you include the elements listed in 505(o) and 21 CFR 314.81(b)(2)(vii). We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies or clinical trials required under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

POSTMARKETING COMMITMENTS SUBJECT TO THE REPORTING REQUIREMENTS UNDER SECTION 506B

We remind you of your postmarketing commitments in your submission dated January 18, 2010. These commitment(s) are listed below.

1585-002 Conduct a prospective, randomized trial evaluating the efficacy and safety of Cayston versus TOBI® (tobramycin solution for inhalation) in the treatment of patients with cystic fibrosis. Enrolled patients should receive 75 mg of aztreonam for inhalation three times daily or 300 mg of tobramycin solution for inhalation twice daily in 28-day treatment cycles over a trial period of 24 weeks. The trial should enroll CF patients ≥ 6 years of age with history of *Pseudomonas aeruginosa* on sputum culture.

Final Protocol Submission: April 13, 2009
Trial Completion Date: 05/2010
Final Report Submission: 09/2010

1585-003 Conduct a prospective trial comparing twice daily and three times daily administration of Cayston to evaluate the presence or absence of a regimen effect on efficacy. The trial should enroll CF patients ≥ 6 years of age with history of *Pseudomonas aeruginosa* on sputum culture.

Final Protocol Submission: 07/2010
Trial Completion Date: 04/2013
Final Report Submission: 01/2014

Submit clinical protocols to your IND 64,402 for this product. Submit nonclinical and chemistry, manufacturing, and controls protocols and all final reports to this NDA. In addition, under 21 CFR 314.81(b)(2)(vii) and 314.81(b)(2)(viii), you should include a status summary of

each commitment in your annual report to this NDA. The status summary should include expected summary completion and final report submission dates, any changes in plans since the last annual report, and, for clinical trials, number of patients entered into each trial. Prominently identify all submissions, including supplements, with the following wording in bold capital letters at the top of the first page of the submission, as appropriate:

- **POSTMARKETING COMMITMENT PROTOCOL**
- **POSTMARKETING COMMITMENT FINAL REPORT**
- **POSTMARKETING COMMITMENT CORRESPONDENCE**

CONTENT OF LABELING

As soon as possible, but no later than 14 days from the date of this letter, please submit the content of labeling [21 CFR 314.50(l)] in structured product labeling (SPL) format, as described at http://www.fda.gov/oc/datacouncil/spl.html, that is identical to the enclosed labeling submitted on February 11, 2010. For administrative purposes, please designate this submission, "**SPL for approved NDA 50-814.**"

CARTON AND IMMEDIATE CONTAINER LABELS

Submit final printed carton and container labels that are identical to the carton and immediate container labels submitted on October 15, 2009, as soon as they are available, but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled *Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (October 2005)*. Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission "**Final Printed Carton and Container Labels for approved NDA 50-814.**" Approval of this submission by FDA is not required before the labeling is used.

Marketing the product with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications
5901-B Ammendale Road
Beltsville, MD 20705-1266

As required under 21 CFR 314.81(b)(3)(i), you must submit final promotional materials, and the package insert, at the time of initial dissemination or publication, accompanied by a Form FDA 2253. For instruction on completing the Form FDA 2253, see page 2 of the Form. For more information about submission of promotional materials to the Division of Drug Marketing, Advertising, and Communications (DDMAC), see http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm.

REPORTING REQUIREMENTS

We remind you that you must comply with reporting requirements for an approved NDA (21 CFR 314.80 and 314.81).

If you have any questions, call Kyong Hyon, Regulatory Project Manager, at (301) 796-0734.

Sincerely,

{See appended electronic signature page}

Katherine A. Laessig, M.D.
Deputy Director
Division of Anti-Infective and Ophthalmology Products
Office of Antimicrobial Products
Center for Drug Evaluation and Research

Enclosure

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-50814	ORIG-1	GILEAD SCIENCES INC	CAYSTON(AZTREONAM FOR INHALATION SOL)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

KATHERINE A LAESSIG
02/22/2010

EXHIBIT 3

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use CAYSTON safely and effectively. See full prescribing information for CAYSTON.

CAYSTON® (aztreonam for inhalation solution)
Initial U.S. Approval: 1986

To reduce the development of drug-resistant bacteria and maintain the effectiveness of CAYSTON and other antibacterial drugs, CAYSTON should be used only to treat patients with cystic fibrosis (CF) known to have *Pseudomonas aeruginosa* in the lungs.

----------INDICATIONS AND USAGE----------

CAYSTON is a monobactam antibacterial indicated to improve respiratory symptoms in cystic fibrosis (CF) patients with *Pseudomonas aeruginosa*. Safety and effectiveness have not been established in pediatric patients below the age of 7 years, patients with FEV_1 <25% or >75% predicted, or patients colonized with *Burkholderia cepacia*. (1)

----------DOSAGE AND ADMINISTRATION----------

- Administer one dose (one single use vial and one ampule of diluent) 3 times a day for 28 days. (2.1)
- Use dose immediately after reconstitution. (2.2)
- Administer only with the Altera® Nebulizer System. Do not administer with any other type of nebulizer. (2.3)

----------DOSAGE FORMS AND STRENGTHS----------

- Lyophilized aztreonam (75 mg/vial) (3)
- Diluent (0.17% sodium chloride): 1 mL/ampule (3)

----------CONTRAINDICATIONS----------

- Do not administer to patients with a known allergy to aztreonam. (4)

----------WARNINGS AND PRECAUTIONS----------

- Allergic reaction to CAYSTON was seen in clinical trials. Stop treatment if an allergic reaction occurs. Use caution when CAYSTON is administered to patients with a known allergic reaction to beta-lactams. (5.1)
- Bronchospasm has been reported with CAYSTON. Stop treatment if chest tightness develops during nebulizer use. (5.2)

----------ADVERSE REACTIONS----------

- Common adverse reactions (more than 5%) occurring more frequently in CAYSTON patients are cough, nasal congestion, wheezing, pharyngolaryngeal pain, pyrexia, chest discomfort, abdominal pain and vomiting. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Gilead Sciences, Inc. at 1-800-GILEAD5, option 3 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See 17 for PATIENT COUNSELING INFORMATION and FDA-Approved Patient Labeling

Revised: February 2010

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed

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1 INDICATIONS AND USAGE

CAYSTON® is indicated to improve respiratory symptoms in cystic fibrosis (CF) patients with *Pseudomonas aeruginosa.* Safety and effectiveness have not been established in pediatric patients below the age of 7 years, patients with FEV_1 <25% or >75% predicted, or patients colonized with *Burkholderia cepacia* [*see Clinical Studies (14)*].

To reduce the development of drug-resistant bacteria and maintain the effectiveness of CAYSTON and other antibacterial drugs, CAYSTON should be used only to treat patients with CF known to have *Pseudomonas aeruginosa* in the lungs.

2 DOSAGE AND ADMINISTRATION

2.1 Dosing Information

The recommended dose of CAYSTON for both adults and pediatric patients 7 years of age and older is one single-use vial (75 mg of aztreonam) reconstituted with 1 mL of sterile diluent administered 3 times a day for a 28-day course (followed by 28 days off CAYSTON therapy). Dosage is not based on weight or adjusted for age. Doses should be taken at least 4 hours apart.

CAYSTON is administered by inhalation using an Altera® Nebulizer System. Patients should use a bronchodilator before administration of CAYSTON.

2.2 Instructions for CAYSTON Reconstitution

CAYSTON should be administered immediately after reconstitution. Do not reconstitute CAYSTON until ready to administer a dose.

Take one amber glass vial containing CAYSTON and one diluent ampule from the carton. To open the glass vial, carefully remove the metal ring by pulling the tab and remove the gray rubber stopper. Twist the tip off the diluent ampule and squeeze the liquid into the glass vial. Replace the rubber stopper, then gently swirl the vial until contents have completely dissolved.

The empty vial, stopper, and diluent ampule should be disposed of properly upon completion of dosing.

2.3 Instructions for CAYSTON Administration

CAYSTON is administered by inhalation using an Altera Nebulizer System. CAYSTON should not be administered with any other nebulizer. CAYSTON should not be mixed with any other drugs in the Altera Nebulizer Handset.

CAYSTON is not for intravenous or intramuscular administration.

Patients should use a bronchodilator before administration of CAYSTON. Short-acting bronchodilators can be taken between 15 minutes and 4 hours prior to each dose of CAYSTON. Alternatively, long-acting bronchodilators can be taken between 30 minutes and 12 hours prior to administration of CAYSTON. For patients taking multiple inhaled therapies, the recommended order of administration is as follows: bronchodilator, mucolytics, and lastly, CAYSTON.

To administer CAYSTON, pour the reconstituted solution into the handset of the nebulizer system. Turn the unit on. Place the mouthpiece of the handset in your mouth and breathe normally only through your mouth. Administration typically takes between 2 and 3 minutes. Further patient instructions on how to administer CAYSTON are provided in the FDA-approved patient labeling. Instructions on testing nebulizer functionality and cleaning the handset are provided in the Instructions for Use included with the nebulizer system.

3 DOSAGE FORMS AND STRENGTHS

A dose of CAYSTON consists of a single-use vial of sterile, lyophilized aztreonam (75 mg) reconstituted with a 1 mL ampule of sterile diluent (0.17% sodium chloride). Reconstituted CAYSTON is administered by inhalation.

4 CONTRAINDICATIONS

CAYSTON is contraindicated in patients with a known allergy to aztreonam.

5 WARNINGS AND PRECAUTIONS

5.1 Allergic Reactions

Severe allergic reactions have been reported following administration of aztreonam for injection to patients with no known history of exposure to aztreonam. In addition, allergic reaction with facial rash, facial swelling, and throat tightness was

reported with CAYSTON in clinical trials. If an allergic reaction to CAYSTON occurs, stop administration of CAYSTON and initiate treatment as appropriate.

Caution is advised when administering CAYSTON to patients if they have a history of beta-lactam allergy, although patients with a known beta-lactam allergy have received CAYSTON in clinical trials and no severe allergic reactions were reported. A history of allergy to beta-lactam antibiotics, such as penicillins, cephalosporins, and/or carbapenems, may be a risk factor, since cross-reactivity may occur.

5.2 Bronchospasm

Bronchospasm is a complication associated with nebulized therapies, including CAYSTON. Reduction of 15% or more in forced expiratory volume in 1 second (FEV_1) immediately following administration of study medication after pretreatment with a bronchodilator was observed in 3% of patients treated with CAYSTON.

5.3 Decreases in FEV_1 After 28-Day Treatment Cycle

In clinical trials, patients with increases in FEV_1 during a 28-day course of CAYSTON were sometimes treated for pulmonary exacerbations when FEV_1 declined after the treatment period. Healthcare providers should consider a patient's baseline FEV_1 measured prior to CAYSTON therapy and the presence of other symptoms when evaluating whether post-treatment changes in FEV_1 are caused by a pulmonary exacerbation.

5.4 Development of Drug-Resistant Bacteria

Prescribing CAYSTON in the absence of known *Pseudomonas aeruginosa* infection in patients with CF is unlikely to provide benefit and increases the risk of development of drug-resistant bacteria.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of drugs cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety of CAYSTON was evaluated in 344 patients from two placebo-controlled trials and one open-label follow-on trial. In controlled trials, 146 patients with CF received 75 mg CAYSTON 3 times a day for 28 days.

Table 1 displays adverse reactions reported in more than 5% of patients treated with CAYSTON 3 times a day in placebo-controlled trials. The listed adverse reactions occurred more frequently in CAYSTON-treated patients than in placebo-treated patients.

Table 1. Adverse Reactions Reported in more than 5% of Patients Treated with CAYSTON in the Placebo-Controlled Trials

Event (Preferred Term)	Placebo (N = 160) n (%)	CAYSTON 75 mg 3 times a day (N = 146) n (%)
Cough	82 (51%)	79 (54%)
Nasal congestion	19 (12%)	23 (16%)
Wheezing	16 (10%)	23 (16%)
Pharyngolaryngeal pain	17 (11%)	18 (12%)
Pyrexia	9 (6%)	19 (13%)
Chest discomfort	10 (6%)	11 (8%)
Abdominal Pain	8 (5%)	10 (7%)
Vomiting	7 (4%)	9 (6%)

Adverse reactions that occurred in less than 5% of patients treated with CAYSTON were bronchospasm (3%) [*see Warnings and Precautions (5.2)*] and rash (2%).

7 DRUG INTERACTIONS

No formal clinical studies of drug interactions with CAYSTON have been conducted.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B

No reproductive toxicology studies have been conducted with CAYSTON. However, studies were conducted with aztreonam for injection. Aztreonam has been shown to cross the placenta and enter fetal circulation. No evidence of embryo or fetotoxicity or

teratogenicity has been shown in studies with pregnant rats and rabbits. In rats receiving aztreonam for injection during late gestation and lactation, no drug induced changes in maternal, fetal or neonatal parameters were observed. These animal reproduction and developmental toxicity studies used parenteral routes of administration that would provide systemic exposures far in excess of the average peak plasma levels measured in humans following CAYSTON therapy.

No adequate and well-controlled studies of aztreonam for injection or CAYSTON in pregnant women have been conducted. Because animal reproduction studies are not always predictive of human response, CAYSTON should be used during pregnancy only if clearly needed.

8.3 Nursing Mothers

Following administration of aztreonam for injection, aztreonam is excreted in human milk at concentrations that are less than one percent of those determined in simultaneously obtained maternal serum. Peak plasma concentrations of aztreonam following administration of CAYSTON (75 mg) are approximately 1% of peak concentrations observed following IV aztreonam (500 mg). Therefore, use of CAYSTON during breastfeeding is unlikely to pose a risk to infants.

8.4 Pediatric Use

Patients 7 years and older were included in clinical trials with CAYSTON. Fifty-five patients under 18 years of age received CAYSTON in placebo-controlled trials. No dose adjustments were made for pediatric patients. Pyrexia was more commonly reported in pediatric patients than in adult patients. Safety and effectiveness in pediatric patients below the age of 7 years have not been established.

8.5 Geriatric Use

Clinical trials of CAYSTON did not include CAYSTON-treated patients aged 65 years of age and older to determine whether they respond differently from younger patients.

8.6 Use in Patients with Renal Impairment

Aztreonam is known to be excreted by the kidney. Placebo-controlled clinical trials with CAYSTON excluded patients with abnormal baseline renal function (defined as serum creatinine greater than 2 times the upper limit of normal range). Given the low systemic

exposure of aztreonam following administration of CAYSTON, clinically relevant accumulation of aztreonam is unlikely to occur in patients with renal impairment. Therefore, CAYSTON may be administered to patients with mild, moderate and severe renal impairment with no dosage adjustment.

10 OVERDOSAGE

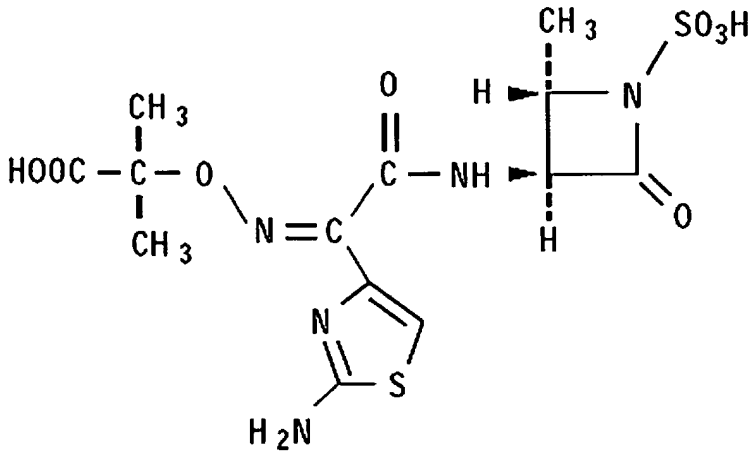
No overdoses have been reported with CAYSTON in clinical trials to date. In clinical trials, 225 mg doses of CAYSTON via inhalation were associated with higher rates of drug-related respiratory adverse reactions, particularly cough. Since the peak plasma concentration of aztreonam following administration of CAYSTON (75 mg) is approximately 0.6 mcg/mL, compared to a serum concentration of 54 mcg/mL following administration of aztreonam for injection (500 mg), no systemic safety issues associated with CAYSTON overdose are anticipated.

11 DESCRIPTION

A dose of CAYSTON consists of a 2 mL amber glass vial containing lyophilized aztreonam (75 mg) and lysine (46.7 mg), and a low-density polyethylene ampule containing 1 mL sterile diluent (0.17% sodium chloride). The reconstituted solution is for inhalation. The formulation contains no preservatives or arginine.

The active ingredient in CAYSTON is aztreonam, a monobactam antibacterial. The monobactams are structurally different from beta-lactam antibiotics (e.g., penicillins, cephalosporins, carbapenems) due to a monocyclic nucleus. This nucleus contains several side chains; sulfonic acid in the 1-position activates the nucleus, an aminothiazolyl oxime side chain in the 3-position confers specificity for aerobic Gram-negative bacteria including *Pseudomonas spp.*, and a methyl group in the 4-position enhances beta-lactamase stability.

Aztreonam is designated chemically as (*Z*)-2-[[[(2-amino-4-thiazolyl)[[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl]methylene]amino]oxy]-2-methylpropionic acid. The structural formula is presented below:



CAYSTON is a white to off-white powder. CAYSTON is sterile, hygroscopic, and light sensitive. Once reconstituted with the supplied diluent, the pH range is 4.5 to 6.0.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Aztreonam is an antibacterial drug [*see Clinical Pharmacology (12.4)*].

12.3 Pharmacokinetics

Sputum Concentrations

Sputum aztreonam concentrations exhibited considerable variability between patients receiving CAYSTON (75 mg) in clinical trials. The mean sputum concentration 10 minutes following the first dose of CAYSTON (n = 195 patients with CF) was 726 mcg/g. Mean sputum concentrations of aztreonam in patients receiving CAYSTON 3 times a day for 28 days were 984 mcg/g, 793 mcg/g, and 715 mcg/g 10 minutes after dose administration on Days 0, 14, and 28, respectively, indicating no accumulation of aztreonam in sputum.

Plasma Concentrations

Plasma aztreonam concentrations exhibited considerable variability between patients receiving CAYSTON (75 mg) in the clinical trials. The mean plasma concentration one hour following the first dose of CAYSTON (at approximately the peak plasma concentration) was 0.59 mcg/mL. Mean peak plasma concentrations in patients receiving CAYSTON 3 times a day for 28 days were 0.55 mcg/mL, 0.67 mcg/mL, and 0.65 mcg/mL on Days 0, 14, and 28, respectively,

indicating no systemic accumulation of aztreonam. In contrast, the serum concentration of aztreonam following administration of aztreonam for injection (500 mg) is approximately 54 mcg/mL.

Absorption

Evaluation of plasma and urine aztreonam concentrations following administration of CAYSTON indicates low systemic absorption of aztreonam. Approximately 10% of the total CAYSTON dose is excreted in the urine as unchanged drug, as compared to 60–65% following intravenous administration of aztreonam for injection.

Distribution

The protein binding of aztreonam in serum is approximately 56% and is independent of dose.

Metabolism

Following intramuscular administration of aztreonam for injection 500 mg every 8 hours for 7 days, approximately 6% of the dose was excreted as a microbiologically inactive open β-lactam ring hydrolysis product in an 8-hour urine collection on the last day of multiple dosing.

Excretion

The elimination half-life of aztreonam from plasma is approximately 2.1 hours following administration of CAYSTON to adult patients with CF, similar to what has been reported for aztreonam for injection. Approximately 10% of the total CAYSTON dose is excreted in the urine as unchanged drug. Systemically absorbed aztreonam is eliminated about equally by active tubular secretion and glomerular filtration. Following administration of a single intravenous dose of radiolabeled aztreonam for injection, about 12% of the dose was recovered in the feces.

12.4 Microbiology

Mechanism of Action

Aztreonam exhibits activity *in vitro* against Gram-negative aerobic pathogens including *P. aeruginosa*. Aztreonam binds to penicillin-binding proteins of susceptible bacteria, which leads to inhibition of bacterial cell wall synthesis and death of the cell. Aztreonam activity is not decreased in the presence of CF lung secretions.

Susceptibility Testing

A single sputum sample from a patient with CF may contain multiple morphotypes of *P. aeruginosa* and each morphotype may have a different level of *in vitro* susceptibility to aztreonam. There are no *in vitro* susceptibility test interpretive criteria for isolates of *P. aeruginosa* obtained from the sputum of CF patients.[1]

Development of Resistance

No changes in the susceptibility of *P. aeruginosa* to aztreonam were observed following a 28-day course of CAYSTON in the placebo-controlled trials.

Cross-Resistance

No cross-resistance to other classes of antibiotics, including aminoglycosides, quinolones, and beta-lactams, was observed following a 28-day course of CAYSTON in the Phase 3 placebo-controlled trials or in an open-label follow-on trial of up to nine 28-day courses of 75 mg CAYSTON 3 times a day.

Other

No trends in the treatment-emergent isolation of other bacterial respiratory pathogens (*Burkholderia cepacia, Stenotrophomonas maltophilia, Achromobacter xylosoxidans*, and *Staphylococcus aureus*) were observed in clinical trials. There was a slight increase in the isolation of *Candida spp.* following up to nine 28-day courses of CAYSTON therapy.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

A 104-week rat inhalation toxicology study to assess the carcinogenic potential of aztreonam demonstrated no drug-related increase in the incidence of tumors. Rats were exposed to aztreonam for up to 4 hours per day. Peak plasma levels of aztreonam averaging approximately 6.8 mcg/mL were measured in rats at the highest dose level. This is approximately 12-fold higher than the average peak plasma level measured in humans following CAYSTON therapy.

Genetic toxicology studies performed *in vitro* demonstrated that aztreonam did not induce structural chromosome aberrations in CHO cells and did not induce mutations at the TK locus in mouse lymphoma L5178Y $TK^{+/-}$ cells. Likewise, genetic toxicology

studies performed *in vivo* did not reveal evidence of mutagenic potential.

Aztreonam did not impair the fertility of rats when administered at doses that would provide systemic exposures far in excess of peak plasma levels measured in humans following CAYSTON therapy.

14 CLINICAL STUDIES

CAYSTON was evaluated over a period of 28 days of treatment in a randomized, double-blind, placebo-controlled, multicenter trial that enrolled patients with CF and *P. aeruginosa*. This trial was designed to evaluate improvement in respiratory symptoms. Patients 7 years of age and older and with FEV_1 of 25% to 75% predicted were enrolled. All patients received CAYSTON or placebo on an outpatient basis administered with the Altera Nebulizer System. All patients were required to take a dose of an inhaled bronchodilator (beta-agonist) prior to taking a dose of CAYSTON or placebo. Patients were receiving standard care for CF, including drugs for obstructive airway diseases.

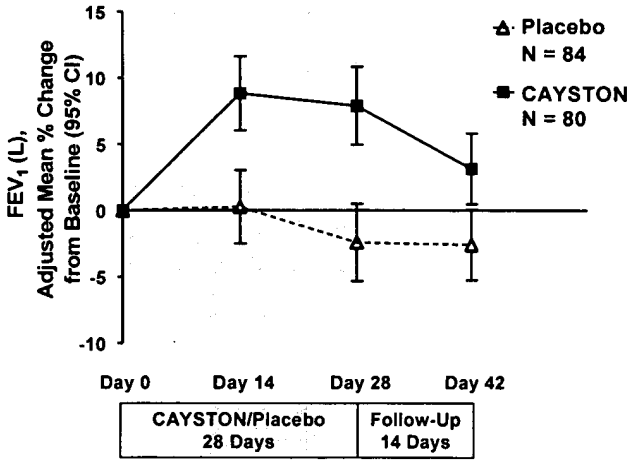
The trial enrolled 164 patients with CF and *P. aeruginosa*. The mean age was 30 years, and the mean baseline FEV_1 % predicted was 55%; 43% were females and 96% were Caucasian. These patients were randomized in a 1:1 ratio to receive either CAYSTON (75 mg) or volume-matched placebo administered by inhalation 3 times a day for 28 days. Patients were required to have been off antibiotics for at least 28 days before treatment with study drug. The primary efficacy endpoint was improvement in respiratory symptoms on the last day of treatment with CAYSTON or placebo. Respiratory symptoms were also assessed two weeks after the completion of treatment with CAYSTON or placebo. Changes in respiratory symptoms were assessed using a questionnaire that asks patients to report on symptoms like cough, wheezing, and sputum production.

Improvement in respiratory symptoms was noted for CAYSTON-treated patients relative to placebo-treated patients on the last day of drug treatment. Statistically significant improvements were seen in both adult and pediatric patients, but were substantially smaller in adult patients. Two weeks after completion of treatment, a difference in respiratory symptoms between treatment groups was still present, though the difference was smaller.

Pulmonary function, as measured by FEV_1 (L), increased from baseline in patients treated with CAYSTON (see Figure 1). The treatment difference at Day 28 between CAYSTON-treated and

placebo-treated patients for percent change in FEV_1 (L) was statistically significant at 10% (95% CI: 6%, 14%). Improvements in FEV_1 were comparable between adult and pediatric patients. Two weeks after completion of drug treatment, the difference in FEV_1 between CAYSTON and placebo groups had decreased to 6% (95% CI: 2%, 9%).

Figure 1. Adjusted Mean Percent Change in FEV_1 from Baseline to Study End (Days 0-42).



15 REFERENCES

1. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Eighth Edition; Approved Standard. CLSI Document M7-A8. CLSI, Wayne, PA 19087. January, 2009.

16 HOW SUPPLIED/STORAGE AND HANDLING

Each kit for a 28-day course of CAYSTON contains 84 sterile vials of CAYSTON and 88 ampules of sterile diluent packed in 2 cartons, each carton containing a 14-day supply. The four additional diluent ampules are provided in case of spillage.

Package Configuration	Dosage Strength	NDC No.
28-Day Kit	75 mg	61958-0901-1

CAYSTON vials and diluent ampules should be stored in the refrigerator at 2 °C to 8 °C (36 °F to 46 °F) until needed. Once removed from the refrigerator, CAYSTON and diluent may be stored at room temperature (up to 25 °C/77 °F) for up to 28 days. Do not separate the CAYSTON vials from the diluent ampules. CAYSTON should be protected from light.

Do not use CAYSTON if it has been stored at room temperature for more than 28 days. Do not use CAYSTON beyond the expiration date stamped on the vial. Do not use diluent beyond the expiration date embossed on the ampule.

CAYSTON should be used immediately upon reconstitution. Do not reconstitute more than one dose at a time.

Do not use diluent or reconstituted CAYSTON if it is cloudy or if there are particles in the solution.

17 PATIENT COUNSELING INFORMATION

See FDA-Approved Patient Labeling

Patients should be advised that CAYSTON is for inhalation use only and that CAYSTON should only be administered using the Altera Nebulizer System. Patients should be instructed only to reconstitute CAYSTON with the provided diluent and not mix other drugs with CAYSTON in the Altera Nebulizer System.

Patients should be advised to complete the full 28-day course of CAYSTON even if they are feeling better. Inform the patient that if they miss a dose, they should take all 3 daily doses as long as the doses are at least 4 hours apart.

Patients should be advised to use a bronchodilator prior to administration of CAYSTON. Patients taking several inhaled medications should be advised to use the medications in the following order of administration: bronchodilator, mucolytics, and lastly, CAYSTON.

Patients should be advised to tell their doctor if they have new or worsening symptoms. Patients who believe they are experiencing an allergic reaction to CAYSTON should be advised to contact their doctor immediately.

Patients should be counseled that antibacterial drugs including CAYSTON should only be used to treat bacterial infections. They do not treat viral infection (e.g., the common cold). When CAYSTON is prescribed to treat a bacterial infection, patients should be told that although it is common to feel better early in the course of therapy, the medication should be taken as directed. Skipping doses or not completing the full course of therapy may (1) decrease the effectiveness of the immediate treatment and (2) increase the likelihood that bacteria will develop resistance and will not be treatable by CAYSTON or other antibacterial drugs in the future.

Manufactured by: Gilead Sciences, Inc., Foster City, CA 94404

50-814-DGS-002

FDA-Approved Patient Labeling

Patient Information

CAYSTON® (kay-stun)
(aztreonam for inhalation solution)

Read this Patient Information before you start taking CAYSTON and each time you get a refill. This information does not take the place of talking with your doctor about your medical condition or your treatment.

What is CAYSTON?
CAYSTON is a prescription inhaled antibiotic. CAYSTON is used to improve breathing symptoms in people with cystic fibrosis (CF) who have *Pseudomonas aeruginosa (P. aeruginosa)* in their lungs.

CAYSTON is only for infections caused by bacteria. It is not for infections caused by viruses, such as the common cold.

CAYSTON is used only with the Altera® Nebulizer System.

It is not known if CAYSTON is safe and effective in children under the age of 7.

Who should not take CAYSTON?
Do not take CAYSTON if you are allergic to aztreonam (AZACTAM®).

What should I tell my doctor before taking CAYSTON?
Before taking CAYSTON, tell your doctor if you:

- are allergic to any antibiotics.
- are pregnant or plan to become pregnant.
- are breast-feeding or plan to breast feed. Talk to your doctor about the best way to breast feed your baby if you take CAYSTON.

Tell your doctor about all the medicine you take, including prescription and non-prescription medicines, vitamins and herbal supplements.

Know the medicines you take. Keep a list of them to show your doctor and pharmacist when you get a new medicine.

How should I take CAYSTON?

- Take CAYSTON exactly as prescribed by your doctor.

- The dose of CAYSTON for both adults and children 7 years of age and older is one vial of CAYSTON, mixed with one ampule of saline (diluent) 3 times a day.
- Doses of CAYSTON should be taken at least 4 hours apart (for example: morning, after school, and before bed).
- CAYSTON should be taken for 28 days.
- CAYSTON is taken as a breathing treatment (inhalation) with the Altera Nebulizer System. Do not use any other nebulizer for your CAYSTON treatment.
- You should use an inhaled bronchodilator (a type of medicine used to relax and open your airways) before taking a dose of CAYSTON. If you do not have an inhaled bronchodilator, ask your doctor to prescribe one for you.
- If you are taking several medicines or treatments to treat your cystic fibrosis, you should take your medicines or other treatments in this order:
    1) bronchodilator
    2) mucolytics (medicines to help clear mucus from your lungs)
    3) CAYSTON
- You should take CAYSTON as prescribed, in courses of 28 days on CAYSTON, followed by at least 28 days off CAYSTON, as directed by your doctor.
- Do not mix CAYSTON with any other medicines in your Altera Nebulizer System.
- Do not mix CAYSTON with the saline until right before you are ready to use it. Do not mix more than one dose of CAYSTON at a time.
- Each treatment should take about 2 to 3 minutes.
- If you miss a dose of CAYSTON, you can still take all 3 daily doses as long as they are at least 4 hours apart.
- It is important for you to finish taking the full 28-day course of CAYSTON even if you are feeling better. If you skip doses or do not finish the full 28-day course of CAYSTON, your infection may not be fully treated and CAYSTON may not work as well as a treatment for infections in the future.
- See the end of this Patient Information leaflet for the Patient Instructions for Use on how to take CAYSTON the right way.

What are the possible side effects of CAYSTON?

CAYSTON can cause serious side effects, including:

- **Severe allergic reactions. Stop your treatment with CAYSTON and call your doctor right away if you have any symptoms of an allergic reaction, including:**
    - Rash or swelling of your face
    - Throat tightness

- **Trouble breathing right after treatment with CAYSTON (bronchospasm).** To decrease the chance of this happening, be sure to use your inhaled bronchodilator medicine before each treatment with CAYSTON. See "How should I take CAYSTON?"

Common side effects of CAYSTON include:

- Cough
- Nasal congestion
- Wheezing
- Sore throat
- Fever. Fever may be more common in children than in adults.
- Chest discomfort
- Stomach area (abdominal) pain
- Vomiting

Tell your doctor if you have any new or worsening symptoms while taking CAYSTON. Tell your doctor about any side effect that bothers you or that does not go away.

These are not all the possible side effects of CAYSTON. For more information, ask your doctor or pharmacist.

Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

How should I store CAYSTON?

- Each CAYSTON kit contains enough vials of CAYSTON and ampules of saline for 28 days of treatment. There are 4 extra saline ampules in case some saline spills.
- Always keep your CAYSTON and saline together.
- Store CAYSTON and saline in the refrigerator at 36 °F to 46 °F (2 °C to 8 °C) until needed.
- When you remove CAYSTON and saline from the refrigerator, they may be stored at room temperature (less than 77 °F) for up to 28 days. Do not use any CAYSTON that has been stored at room temperature for more than 28 days.
- Keep CAYSTON away from light.
- Do not use CAYSTON after the expiration date on the vial. Do not use the saline after the expiration date on the ampule.

Keep CAYSTON and all medicines out of the reach of children.

General information about CAYSTON

Medicines are sometimes prescribed for purposes other than those listed in a Patient Information leaflet. Do not use CAYSTON for a condition for which it was not prescribed. Do not give CAYSTON to other people, even if they have the same symptoms that you have. It may harm them.

This Patient Information leaflet summarizes the most important information about CAYSTON. If you would like more information, talk with your doctor. You can ask your pharmacist or doctor for information about CAYSTON that is written for health professionals.

For more information, call 1-877-7CAYSTON (1-877-722-9786).

What are the ingredients in CAYSTON?
Active ingredient: aztreonam
Inactive ingredient: sodium chloride (diluent)

Patient Instructions for Use

CAYSTON®
(aztreonam for inhalation solution)

Be sure that you read, understand and follow the Patient Instructions for Use below for the right way to take CAYSTON. If you have any questions, ask your doctor or pharmacist.

You will need the following supplies (Figure 1):

- 1 amber colored CAYSTON vial
- 1 ampule of saline (diluent)
- Altera Nebulizer System

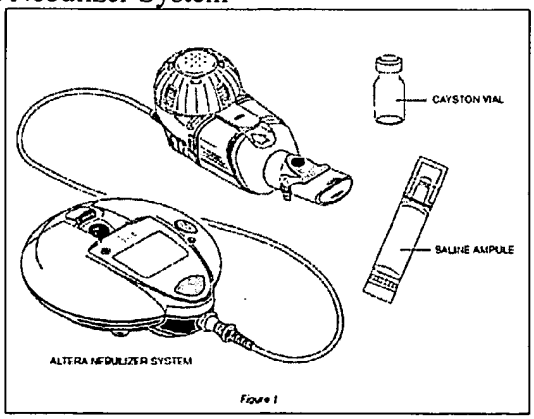


Figure 1

Check to make sure that your Altera Nebulizer System works properly before starting your treatment with CAYSTON. See the manufacturer's instructions for use that comes with your Altera Nebulizer System. This should have complete information about how to put together (assemble), prepare, use, and care for your Altera Nebulizer System.

Step 1 Preparing your CAYSTON for inhalation

1. Mix (reconstitute) CAYSTON with the saline only when ready to take a dose. Take one amber vial of CAYSTON and one ampule of saline from the carton. Separate the saline ampules by gently pulling apart.

2. Look at the ampule of saline. If it looks cloudy do not use it. Throw away this ampule and get another ampule of saline.

3. Gently tap the vial so that the powder settles to the bottom of the vial. This helps you get the proper dose of medicine. Open the amber drug vial by lifting up the metal flap on the top (Figure 2) and pulling down (Figure 3) to carefully remove the entire metal ring from the vial (Figure 4). Safely dispose of the ring in household garbage. Carefully remove the rubber stopper.

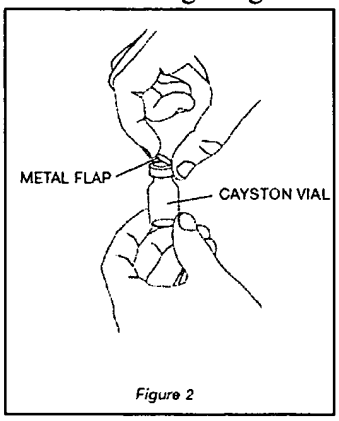


Figure 2

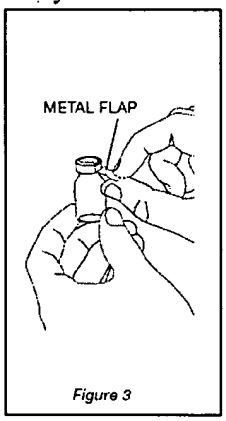


Figure 3

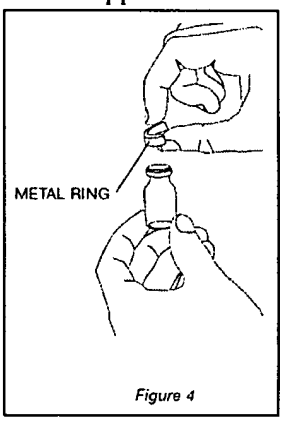


Figure 4

4. Open the ampule of saline by twisting off the tip. Squeeze out the contents completely into the vial (Figure 5). Next, close the vial with the rubber stopper and gently swirl the vial until the powder has completely dissolved and the liquid is clear.

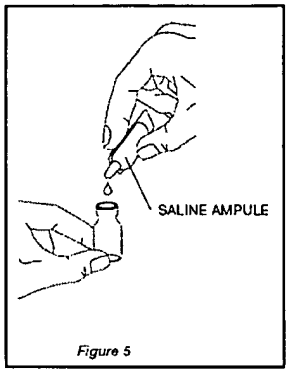


Figure 5

5. After mixing CAYSTON with the saline, check to make sure the diluted medicine is clear. If it is cloudy or has particles in it, do not use this medicine. Throw away this dose of medicine and start over again with a new vial of CAYSTON and a new ampule of saline.

6. Use CAYSTON right away after you mix with the saline.

Step 2 Taking your CAYSTON treatment

See the manufacturer's instructions for use that comes with your Altera Nebulizer System for complete instructions on taking a treatment, and how to clean and disinfect your Altera Nebulizer Handset.

7. Make sure the handset is on a flat, stable surface.

8. Remove the rubber stopper from the vial, then pour all of the mixed CAYSTON and saline into the Medication Reservoir of the handset (Figure 6). Be sure to completely empty the vial, gently tapping the vial against the side of the Medication Reservoir if necessary. Close the Medication Reservoir (Figure 7).

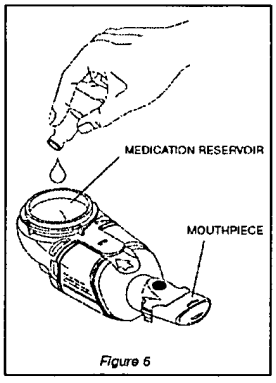


Figure 6

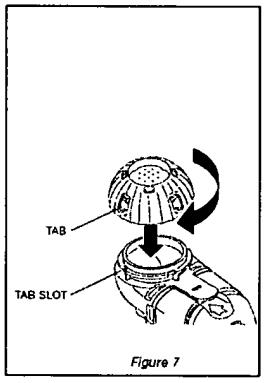
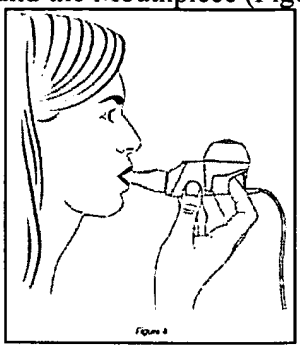


Figure 7

9. Begin your treatment by sitting in a relaxed, upright position. Hold the handset level, and place the Mouthpiece in your mouth. Close your lips around the Mouthpiece (Figure 8).



10. Breathe in and out normally (inhale and exhale) through the Mouthpiece. **Avoid breathing through your nose.** Continue to inhale and exhale comfortably until the treatment is finished.

11. The empty vial, stopper and saline ampule should be disposed of in household garbage upon completion of dosing.

Manufactured by: Gilead Sciences, Inc., Foster City, CA 94404

50-814-DGS-002

EXHIBIT 4

Bristol-Myers Squibb Company

élan

Rx only

AZACTAM®

(aztreonam for injection, USP)

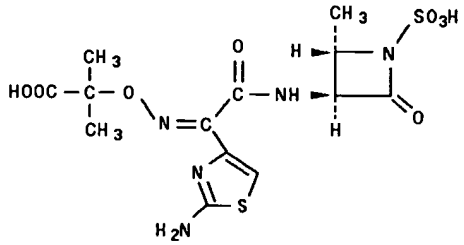
To reduce the development of drug-resistant bacteria and maintain the effectiveness of AZACTAM® and other antibacterial drugs, AZACTAM should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria.

DESCRIPTION

AZACTAM® (aztreonam for injection, USP) contains the active ingredient aztreonam, a monobactam. It was originally isolated from *Chromobacterium violaceum*. It is a synthetic bactericidal antibiotic.

The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics (eg, penicillins, cephalosporins, cephamycins). The sulfonic acid substituent in the 1-position of the ring activates the beta-lactam moiety; an aminothiazolyl oxime side chain in the 3-position and a methyl group in the 4-position confer the specific antibacterial spectrum and beta-lactamase stability.

Aztreonam is designated chemically as (Z)-2-[[[(2-amino-4-thiazolyl)[[(2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl]methylene]amino]oxy]-2-methylpropionic acid. Structural formula:



$C_{13}H_{17}N_5O_8S_2$ MW 435.44

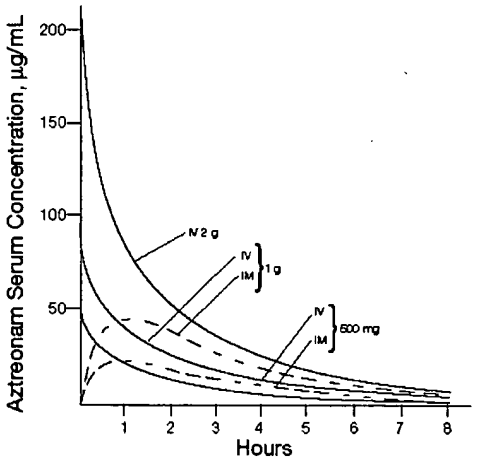
AZACTAM is a sterile, nonpyrogenic, sodium-free, white powder containing approximately 780 mg arginine per gram of aztreonam. Following constitution, the product is for intramuscular or intravenous use. Aqueous solutions of the product have a pH in the range of 4.5 to 7.5.

CLINICAL PHARMACOLOGY

Single 30-minute intravenous infusions of 500 mg, 1 g, and 2 g doses of AZACTAM (aztreonam for injection, USP) in healthy subjects produced aztreonam peak serum levels of 54 µg/mL, 90 µg/mL, and 204 µg/mL, respectively, immediately after administration; at 8 hours, serum levels were 1 µg/mL, 3 µg/mL, and 6 µg/mL, respectively (Figure 1). Single 3-minute intravenous injections of the same doses resulted in serum levels of 58 µg/mL, 125 µg/mL, and 242 µg/mL at 5 minutes following completion of injection.

Serum concentrations of aztreonam in healthy subjects following completion of single intramuscular injections of 500 mg and 1 g doses are depicted in Figure 1; maximum serum concentrations occur at about 1 hour. After identical single intravenous or intramuscular doses of AZACTAM, the serum concentrations of aztreonam are comparable at 1 hour (1.5 hours from start of intravenous infusion) with similar slopes of serum concentrations thereafter.

FIGURE 1



The serum levels of aztreonam following single 500 mg or 1 g (intramuscular or intravenous) or 2 g (intravenous) doses of AZACTAM exceed the MIC_{90} for *Neisseria* sp., *Haemophilus influenzae* and most genera of the *Enterobacteriaceae* for 8 hours (for *Enterobacter* sp., the 8-hour serum levels exceed the MIC for 80% of strains). For *Pseudomonas aeruginosa*, a single 2 g intravenous dose produces serum levels that exceed the MIC_{90} for approximately 4 to 6 hours. All of the above doses of AZACTAM result in average urine levels of aztreonam that exceed the MIC_{90} for the same pathogens for up to 12 hours.

When aztreonam pharmacokinetics were assessed for adult and pediatric patients, they were found to be comparable (down to 9 months old). The serum half-life of aztreonam averaged 1.7 hours (1.5 to 2.0) in subjects with normal renal function, independent of the dose and route of administration. In healthy subjects, based on a 70 kg person, the serum clearance was 91 mL/min and renal clearance was

56 mL/min; the apparent mean volume of distribution at steady-state averaged 12.6 liters, approximately equivalent to extracellular fluid volume.

In elderly patients, the mean serum half-life of aztreonam increased and the renal clearance decreased, consistent with the age-related decrease in creatinine clearance.[1-4] The dosage of AZACTAM should be adjusted accordingly (see **DOSAGE AND ADMINISTRATION: Renal Impairment in Adult Patients**).

In patients with impaired renal function, the serum half-life of aztreonam is prolonged. (See **DOSAGE AND ADMINISTRATION: Renal Impairment in Adult Patients.**) The serum half-life of aztreonam is only slightly prolonged in patients with hepatic impairment since the liver is a minor pathway of excretion.

Average urine concentrations of aztreonam were approximately 1100 μg/mL, 3500 μg/mL, and 6600 μg/mL within the first 2 hours following single 500 mg, 1 g, and 2 g intravenous doses of AZACTAM (30-minute infusions), respectively. The range of average concentrations for aztreonam in the 8- to 12-hour urine specimens in these studies was 25 μg/mL to 120 μg/mL. After intramuscular injection of single 500 mg and 1 g doses of AZACTAM (aztreonam for injection, USP), urinary levels were approximately 500 μg/mL and 1200 μg/mL, respectively, within the first 2 hours, declining to 180 μg/mL and 470 μg/mL in the 6- to 8-hour specimens. In healthy subjects, aztreonam is excreted in the urine about equally by active tubular secretion and glomerular filtration. Approximately 60% to 70% of an intravenous or intramuscular dose was recovered in the urine by 8 hours. Urinary excretion of a single parenteral dose was essentially complete by 12 hours after injection. About 12% of a single intravenous radiolabeled dose was recovered in the feces. Unchanged aztreonam and the inactive beta-lactam ring hydrolysis product of aztreonam were present in feces and urine.

Intravenous or intramuscular administration of a single 500 mg or 1 g dose of AZACTAM every 8 hours for 7 days to healthy subjects produced no apparent accumulation of aztreonam or modification of its disposition characteristics; serum protein binding averaged 56% and was independent of dose. An average of about 6% of a 1 g intramuscular dose was excreted as a microbiologically inactive open beta-lactam ring hydrolysis product (serum half-life approximately 26 hours) of aztreonam in the 0- to 8-hour urine collection on the last day of multiple dosing.

Renal function was monitored in healthy subjects given aztreonam; standard tests (serum creatinine, creatinine clearance, BUN, urinalysis and total urinary protein excretion) as well as special tests (excretion of N-acetyl-β-glucosaminidase, alanine aminopeptidase and β_2-microglobulin) were used. No abnormal results were obtained.

Aztreonam achieves measurable concentrations in the following body fluids and tissues:

EXTRAVASCULAR CONCENTRATIONS OF AZTREONAM AFTER A SINGLE PARENTERAL DOSE[1]

Fluid or Tissue	Dose (g)	Route	Hours Post-injection	Number of Patients	Mean Concentration (μg/mL or μg/g)
Fluids					
bile	1	IV	2	10	39
blister fluid	1	IV	1	6	20
bronchial secretion	2	IV	4	7	5
cerebrospinal fluid (inflamed meninges)	2	IV	0.9-4.3	16	3
pericardial fluid	2	IV	1	6	33
pleural fluid	2	IV	1.1-3.0	3	51
synovial fluid	2	IV	0.8-1.9	11	83
Tissues					
atrial appendage	2	IV	0.9-1.6	12	22
endometrium	2	IV	0.7-1.9	4	9
fallopian tube	2	IV	0.7-1.9	8	12
fat	2	IV	1.3-2.0	10	5
femur	2	IV	1.0-2.1	15	16
gallbladder	2	IV	0.8-1.3	4	23
kidney	2	IV	2.4-5.6	5	67
large intestine	2	IV	0.8-1.9	9	12
liver	2	IV	0.9-2.0	6	47
lung	2	IV	1.2-2.1	6	22
myometrium	2	IV	0.7-1.9	9	11
ovary	2	IV	0.7-1.9	7	13
prostate	1	IM	0.8-3.0	8	8
skeletal muscle	2	IV	0.3-0.7	6	16
skin	2	IV	0.0-1.0	8	25
sternum	2	IV	1	6	6

[1]Tissue penetration is regarded as essential to therapeutic efficacy, but specific tissue levels have not been correlated with specific therapeutic effects.

The concentration of aztreonam in saliva at 30 minutes after a single 1 g intravenous dose (9 patients) was 0.2 μg/mL; in human milk at 2 hours after a single 1 g intravenous dose (6 patients), 0.2 μg/mL, and at 6 hours after a single 1 g intramuscular dose (6 patients), 0.3 μg/mL; in amniotic fluid at 6 to 8 hours after a single 1 g intravenous dose (5 patients), 2 μg/mL. The concentration of aztreonam in peritoneal fluid obtained 1 to 6 hours after multiple 2 g intravenous doses ranged between 12 μg/mL and 90 μg/mL in 7 of 8 patients studied.

Aztreonam given intravenously rapidly reaches therapeutic concentrations in peritoneal dialysis fluid; conversely, aztreonam given intraperitoneally in dialysis fluid rapidly produces therapeutic serum levels.

Concomitant administration of probenecid or furosemide and AZACTAM (aztreonam for injection, USP) causes clinically insignificant increases in the serum levels of aztreonam. Single-dose intravenous pharmacokinetic studies have not shown any significant interaction between aztreonam and concomitantly administered gentamicin, nafcillin sodium, cephradine, clindamycin or metronidazole. No reports of disulfiram-like reactions with alcohol ingestion have been noted; this is not unexpected since aztreonam does not contain a methyl-tetrazole side chain.

Microbiology

Aztreonam exhibits potent and specific activity *in vitro* against a wide spectrum of gram-negative aerobic pathogens including *Pseudomonas aeruginosa.* The bactericidal action of aztreonam results from the inhibition of bacterial cell wall synthesis due to a high affinity of aztreonam for penicillin binding protein 3 (PBP3). Aztreonam, unlike the majority of beta-lactam antibiotics, does not induce beta-lactamase activity and its molecular structure confers a high degree of resistance to hydrolysis by beta-lactamases (ie, penicillinases and cephalosporinases) produced by most gram-negative and gram-positive pathogens; it is, therefore, usually active against gram-negative aerobic microorganisms that are resistant to antibiotics hydrolyzed by beta-lactamases. It is active against many strains that are multiply-resistant to other antibiotics, such as certain cephalosporins, penicillin, and aminoglycosides. Aztreonam maintains its antimicrobial activity over a pH range of 6 to 8 *in vitro*, as well as in the presence of human serum and under anaerobic conditions.

Aztreonam has been shown to be active against most strains of the following microorganisms, both *in vitro* and in clinical infections as described in the **INDICATIONS AND USAGE** section.

Aerobic gram-negative microorganisms:

Citrobacter species, including *C. freundii*
Enterobacter species, including *E. cloacae*
Escherichia coli
Haemophilus influenzae (including ampicillin-resistant and other penicillinase-producing strains)
Klebsiella oxytoca
Klebsiella pneumoniae
Proteus mirabilis
Pseudomonas aeruginosa
Serratia species, including *S. marcescens*

The following *in vitro* data are available, **but their clinical significance is unknown**.

Aztreonam exhibits *in vitro* minimal inhibitory concentrations (MICs) of 8 µg/mL or less against most (≥90%) strains of the following microorganisms; however, the safety and effectiveness of aztreonam in treating clinical infections due to these microorganisms have not been established in adequate and well-controlled clinical trials.

Aerobic gram-negative microorganisms:

Aeromonas hydrophila
Morganella morganii
Neisseria gonorrhoeae (including penicillinase-producing strains)
Pasteurella multocida
Proteus vulgaris
Providencia stuartii
Providencia rettgeri
Yersinia enterocolitica

Aztreonam and aminoglycosides have been shown to be synergistic *in vitro* against most strains of *P. aeruginosa*, many strains of *Enterobacteriaceae*, and other gram-negative aerobic bacilli.

Alterations of the anaerobic intestinal flora by broad spectrum antibiotics may decrease colonization resistance, thus permitting overgrowth of potential pathogens, eg, *Candida* and *Clostridium* species. Aztreonam has little effect on the anaerobic intestinal microflora in *in vitro* studies. *Clostridium difficile* and its cytotoxin were not found in animal models following administration of aztreonam. (See **ADVERSE REACTIONS:** *Gastrointestinal.*)

Susceptibility Tests

Dilution Techniques: Quantitative methods are used to determine antimicrobial minimal inhibitory concentrations (MICs). These MICs provide estimates of the susceptibility of bacteria to antimicrobial compounds. The MICs should be determined using a standardized procedure. Standardized procedures are based on a dilution method[5] (broth or agar) or equivalent with standardized inoculum concentrations and standardized concentrations of aztreonam powder. The MIC values should be interpreted according to the following criteria:

For testing aerobic microorganisms other than *Haemophilus influenzae*:

MIC (µg/mL)	Interpretation
≤8	Susceptible (S)
16	Intermediate (I)
≥32	Resistant (R)

When testing *Haemophilus influenzae*[a]:

MIC (µg/mL)	Interpretation[b]
≤2	Susceptible (S)

a. Interpretative criteria applicable only to tests performed by broth microdilution method using *Haemophilus* Test Medium (HTM).[5]

b. The current absence of data on resistant strains precludes defining any categories other than "Susceptible." Strains yielding MIC results suggestive of a "nonsusceptible" category should be submitted to a reference laboratory for further testing.

A report of "Susceptible" indicates that the pathogen is likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable. A report of "Intermediate" indicates that the result should be considered equivocal, and, if the microorganism is not fully susceptible to alternative, clinically feasible drugs, the test should be repeated. This category implies possible clinical applicability in body sites where the drug is physiologically concentrated or in situations where high dosage of drug can be used. This category also provides a buffer zone which prevents small uncontrolled technical factors from causing major discrepancies in interpretation. A report of "Resistant" indicates that the pathogen is not likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable; other therapy should be selected.

Standardized susceptibility test procedures require the use of laboratory control microorganisms to control the technical aspects of the laboratory procedures. Standard aztreonam powder should provide the following MIC values:

Microorganism	MIC (µg/mL)
Escherichia coli ATCC 25922	0.06-0.25
Haemophilus influenzae[a] ATCC 49247	0.12-0.5

Pseudomonas aeruginosa ATCC 27853	2.0-8.0

a. Range applicable only to tests performed by broth microdilution method using *Haemophilus* Test Medium (HTM).[5]

Diffusion Techniques: Quantitative methods that require measurement of zone diameters also provide reproducible estimates of the susceptibility of bacteria to antimicrobial compounds. One such standardized procedure[6] requires the use of standardized inoculum concentrations. This procedure uses paper disks impregnated with 30 µg aztreonam to test the susceptibility of microorganisms to aztreonam.

Reports from the laboratory providing results of the standard single-disk susceptibility test with a 30 µg aztreonam disk should be interpreted according to the following criteria:

For testing aerobic microorganisms other than *Haemophilus influenzae*:

Zone diameter (mm)	Interpretation
≥22	Susceptible (S)
16 - 21	Intermediate (I)
≤15	Resistant (R)

When testing *Haemophilus influenzae*[a]:

Zone diameter (mm)	Interpretation[b]
≥26	Susceptible (S)

a. Interpretative criteria applicable only to tests performed by disk diffusion method using *Haemophilus* Test Medium (HTM).[6]

b. The current absence of data on resistant strains precludes defining any categories other than "Susceptible." Strains yielding zone diameter results suggestive of a "nonsusceptible" category should be submitted to a reference laboratory for further testing.

Interpretation should be as stated above for results using dilution techniques. Interpretation involves correlation of the diameter obtained in the disk test with the MIC for aztreonam.

As with standardized dilution techniques, diffusion methods require the use of laboratory control microorganisms that are used to control the technical aspects of the laboratory procedures. For the diffusion technique, the 30 µg aztreonam disk should provide the following zone diameters in these laboratory test quality control strains.

Microorganism	Zone diameter (mm)
Escherichia coli ATCC 25922	28-36 mm
Haemophilus influenzae[a] ATCC 49247	30-38 mm
Pseudomonas aeruginosa ATCC 27853	23-29 mm

a. Range applicable only to tests performed by disk diffusion method using *Haemophilus* Test Medium (HTM).[6]

INDICATIONS AND USAGE

To reduce the development of drug-resistant bacteria and maintain the effectiveness of AZACTAM® (aztreonam for injection, USP) and other antibacterial drugs, AZACTAM should be used only to treat or prevent infections that are proven or strongly suspected to be caused by susceptible bacteria. When culture and susceptibility information are available, they should be considered in selecting or modifying antibacterial therapy. In the absence of such data, local epidemiology and susceptibility patterns may contribute to the empiric selection of therapy.

AZACTAM (aztreonam for injection, USP) is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms:

Urinary Tract Infections (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca**, *Citrobacter* species* and *Serratia marcescens**.

Lower Respiratory Tract Infections, including pneumonia and bronchitis caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens**.

Septicemia caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis**, *Serratia marcescens** and *Enterobacter* species.

Skin and Skin-Structure Infections, including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Citrobacter* species*.

Intra-abdominal Infections, including peritonitis caused by *Escherichia coli, Klebsiella* species including *K. pneumoniae, Enterobacter* species including *E. cloacae**, *Pseudomonas aeruginosa, Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**.

Gynecologic Infections, including endometritis and pelvic cellulitis caused by *Escherichia coli, Klebsiella pneumoniae**, *Enterobacter* species* including *E. cloacae** and *Proteus mirabilis**.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy

Concurrent initial therapy with other antimicrobial agents and AZACTAM (aztreonam for injection, USP) is recommended before the causative organism(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected as etiologic agents, therapy should be initiated using an anti-anaerobic agent concurrently with AZACTAM (see **DOSAGE AND ADMINISTRATION**). Certain antibiotics (eg, cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase-inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing of the causative organism(s), appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS

This preparation is contraindicated in patients with known hypersensitivity to aztreonam or any other component in the formulation.

WARNINGS

Both animal and human data suggest that AZACTAM is rarely cross-reactive with other beta-lactam antibiotics and weakly immunogenic. Treatment with aztreonam can result in hypersensitivity reactions in patients with or without prior exposure. (See **CONTRAINDICATIONS.**)

Careful inquiry should be made to determine whether the patient has any history of hypersensitivity reactions to any allergens.

While cross-reactivity of aztreonam with other beta-lactam antibiotics is rare, this drug should be administered with caution to any patient with a history of hypersensitivity to beta-lactams (eg, penicillins, cephalosporins, and/or carbapenems). Treatment with aztreonam can result in hypersensitivity reactions in patients with or without prior exposure to aztreonam. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (eg, maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures. (See **ADVERSE REACTIONS.**)

Clostridium difficile associated diarrhea (CDAD) has been reported with use of nearly all antibacterial agents, including AZACTAM, and may range in severity from mild diarrhea to fatal colitis. Treatment with antibacterial agents alters the normal flora of the colon leading to overgrowth of *C. difficile.*

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*Efficacy for this organism in this organ system was studied in fewer than 10 infections.

C. difficile produces toxins A and B which contribute to the development of CDAD. Hypertoxin-producing strains of *C. difficile* cause increased morbidity and mortality, as these infections can be refractory to antimicrobial therapy and may require colectomy. CDAD must be considered in all patients who present with diarrhea following antibiotic use. Careful medical history is necessary since CDAD has been reported to occur over two months after the administration of antibacterial agents.

If CDAD is suspected or confirmed, ongoing antibiotic use not directed against *C. difficile* may need to be discontinued. Appropriate fluid and electrolyte management, protein supplementation, antibiotic treatment of *C. difficile*, and surgical evaluation should be instituted as clinically indicated.

Rare cases of toxic epidermal necrolysis have been reported in association with aztreonam in patients undergoing bone marrow transplant with multiple risk factors including sepsis, radiation therapy and other concomitantly administered drugs associated with toxic epidermal necrolysis.

PRECAUTIONS

General

Prescribing AZACTAM in the absence of a proven or strongly suspected bacterial infection or a prophylactic indication is unlikely to provide benefit to the patient and increases the risk of the development of drug-resistant bacteria.

In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy.

If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics.

The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms (*Staphylococcus aureus* and *Streptococcus faecalis*) and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Information for Patients

Patients should be counseled that antibacterial drugs including AZACTAM should only be used to treat bacterial infections. They do not treat viral infections (eg, the common cold). When AZACTAM is prescribed to treat a bacterial infection, patients should be told that although it is common to feel better early in the course of therapy, the medication should be taken exactly as directed. Skipping doses or not completing the full course of therapy may (1) decrease the effectiveness of the immediate

treatment and (2) increase the likelihood that bacteria will develop resistance and will not be treatable by AZACTAM or other antibacterial drugs in the future.

Diarrhea is a common problem caused by antibiotics which usually ends when the antibiotic is discontinued. Sometimes after starting treatment with antibiotics, patients can develop watery and bloody stools (with or without stomach cramps and fever) even as late as two or more months after having taken the last dose of the antibiotic. If this occurs, patients should contact their physician as soon as possible.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies in animals have not been performed.

Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level.

Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received 5 times the maximum recommended human dose.

Pregnancy

Pregnancy Category B

Aztreonam crosses the placenta and enters the fetal circulation.

Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal, or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation.

There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers

Aztreonam is excreted in human milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use

The safety and effectiveness of intravenous AZACTAM (aztreonam for injection, USP) have been established in the age groups 9 months to 16 years. Use of AZACTAM in these age groups is supported by evidence from adequate and well-controlled studies of AZACTAM in adults with additional efficacy, safety, and pharmacokinetic data from non-comparative clinical studies in pediatric patients. Sufficient data are not available for pediatric patients under 9 months of age or for the following treatment indications/pathogens: septicemia and skin and skin-structure infections (where the skin infection is believed or known to be due to *H. influenzae* type b). In pediatric patients with cystic fibrosis, higher doses of AZACTAM may be warranted. (See **CLINICAL PHARMACOLOGY, DOSAGE AND ADMINISTRATION,** and **CLINICAL STUDIES.**)

Geriatric Use

Clinical studies of AZACTAM did not include sufficient numbers of subjects aged 65 years and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients.[7-10] In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

In elderly patients, the mean serum half-life of aztreonam increased and the renal clearance decreased, consistent with the age-related decrease in creatinine clearance.[1-4] Since aztreonam is known to be substantially excreted by the kidney, the risk of toxic reactions to this drug may be greater in patients with impaired renal function. Because elderly patients are more likely to have decreased renal function, renal function should be monitored and dosage adjustments made accordingly (see **DOSAGE AND ADMINISTRATION: Renal Impairment in Adult Patients** and **Dosage in the Elderly**).

AZACTAM contains no sodium.

ADVERSE REACTIONS

Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively.

Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1% to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity:

Hypersensitivity—anaphylaxis, angioedema, bronchospasm

Hematologic—pancytopenia, neutropenia, thrombocytopenia, anemia, eosinophilia, leukocytosis, thrombocytosis

Gastrointestinal—abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment. (See **WARNINGS**.)

Dermatologic—toxic epidermal necrolysis (see **WARNINGS**), purpura, erythema multiforme, exfoliative dermatitis, urticaria, petechiae, pruritus, diaphoresis

Cardiovascular—hypotension, transient ECG changes (ventricular bigeminy and PVC), flushing

Respiratory—wheezing, dyspnea, chest pain

Hepatobiliary—hepatitis, jaundice

Nervous System—seizure, confusion, vertigo, paresthesia, insomnia, dizziness

Musculoskeletal—muscular aches

Special Senses—tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing, nasal congestion, halitosis

Other—vaginal candidiasis, vaginitis, breast tenderness

Body as a Whole—weakness, headache, fever, malaise

Pediatric Adverse Reactions

Of the 612 pediatric patients who were treated with AZACTAM in clinical trials, less than 1% required discontinuation of therapy due to adverse events. The following systemic adverse events, regardless of drug relationship, occurred in at least 1% of treated patients in domestic clinical trials: rash (4.3%), diarrhea (1.4%), and fever (1.0%). These adverse events were comparable to those observed in adult clinical trials.

In 343 pediatric patients receiving intravenous therapy, the following local reactions were noted: pain (12%), erythema (2.9%), induration (0.9%), and phlebitis (2.1%). In the US patient population, pain occurred in 1.5% of patients, while each of the remaining three local reactions had an incidence of 0.5%.

The following laboratory adverse events, regardless of drug relationship, occurred in at least 1% of treated patients: increased eosinophils (6.3%), increased platelets (3.6%), neutropenia (3.2%), increased AST (3.8%), increased ALT (6.5%), and increased serum creatinine (5.8%).

In US pediatric clinical trials, neutropenia (absolute neutrophil count less than $1000/mm^3$) occurred in 11.3% of patients (8/71) younger than 2 years receiving 30 mg/kg q6h. AST and ALT elevations to greater than 3 times the upper limit of normal were noted in 15% to 20% of patients aged 2 years or above receiving 50 mg/kg q6h. The increased frequency of these reported laboratory adverse events may be due to either increased severity of illness treated or higher doses of AZACTAM (aztreonam for injection, USP) administered.

Adverse Laboratory Changes

Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were:

Hepatic—elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above).

Hematologic—increases in prothrombin and partial thromboplastin times, positive Coombs' test.

Renal—increases in serum creatinine.

OVERDOSAGE

If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION

Dosage in Adult Patients

AZACTAM may be administered intravenously or by intramuscular injection. Dosage and route of administration should be determined by susceptibility of the causative organisms, severity and site of infection, and the condition of the patient.

The intravenous route is recommended for patients requiring single doses greater than 1 g or those with bacterial septicemia, localized parenchymal abscess (eg, intra-abdominal abscess), peritonitis or other severe systemic or life-threatening infections.

The duration of therapy depends on the severity of infection. Generally, AZACTAM should be continued for at least 48 hours after the patient becomes asymptomatic or evidence of bacterial eradication has been obtained. Persistent infections may require treatment for several weeks. Doses smaller than those indicated should not be used.

Renal Impairment in Adult Patients

Prolonged serum levels of aztreonam may occur in patients with transient or persistent renal insufficiency. Therefore, the dosage of AZACTAM should be halved in patients with estimated creatinine clearances between 10 mL/min/1.73 m^2 and 30 mL/min/1.73 m^2 after an initial loading dose of 1 g or 2 g.

When only the serum creatinine concentration is available, the following formula (based on sex, weight, and age of the patient) may be used to approximate the creatinine clearance (Clcr). The serum creatinine should represent a steady state of renal function.

$$\text{Males: Clcr} = \frac{\text{weight (kg)} \times (140\text{-age})}{72 \times \text{serum creatinine (mg/dL)}}$$

Females: 0.85 x above value

In patients with severe renal failure (creatinine clearance less than 10 mL/min/1.73 m^2), such as those supported by hemodialysis, the usual dose of 500 mg, 1 g or 2 g should be given initially. The maintenance dose should be one-fourth of the usual initial dose given at the usual fixed interval of 6, 8 or 12 hours. For serious or life-threatening infections, in addition to the maintenance doses, one-eighth of the initial dose should be given after each hemodialysis session.

Dosage in the Elderly

Renal status is a major determinant of dosage in the elderly; these patients in particular may have diminished renal function. Serum creatinine may not be an accurate determinant of renal status. Therefore, as with all antibiotics eliminated by the kidneys, estimates of creatinine clearance should be obtained, and appropriate dosage modifications made if necessary.

Dosage in Pediatric Patients

AZACTAM (aztreonam for injection, USP) should be administered intravenously to pediatric patients with normal renal function. There are insufficient data regarding intramuscular administration to pediatric patients or dosing in pediatric patients with renal impairment. (See **PRECAUTIONS: Pediatric Use.**)

AZACTAM DOSAGE GUIDELINES		
Type of Infection	Dose	Frequency (hours)
	ADULTS*	
Urinary tract infections	500 mg or 1 g	8 or 12
Moderately severe systemic infections	1 g or 2 g	8 or 12
Severe systemic or life-threatening infections	2 g	6 or 8
*Maximum recommended dose is 8 g per day.		
	PEDIATRIC PATIENTS**	
Mild to moderate infections	30 mg/kg	8
Moderate to severe infections	30 mg/kg	6 or 8
**Maximum recommended dose is 120 mg/kg/day.		

Because of the serious nature of infections due to *Pseudomonas aeruginosa*, dosage of 2 g every 6 or 8 hours is recommended, at least upon initiation of therapy, in systemic infections caused by this organism in adults.

CLINICAL STUDIES

A total of 612 pediatric patients aged 1 month to 12 years were enrolled in uncontrolled clinical trials of aztreonam in the treatment of serious gram-negative infections, including urinary tract, lower respiratory tract, skin and skin-structure, and intra-abdominal infections.

Preparation Of Parenteral Solutions

General

Upon the addition of the diluent to the container, contents should be shaken **immediately** and **vigorously**. Constituted solutions are not for multiple-dose use; should the entire volume in the container not be used for a single dose, the unused solution must be discarded.

Depending upon the concentration of aztreonam and diluent used, constituted AZACTAM yields a colorless to light straw yellow solution which may develop a slight pink tint on standing (potency is not affected). Parenteral drug products should be inspected visually for particulate matter and discoloration whenever solution and container permit.

Admixtures With Other Antibiotics

Intravenous infusion solutions of AZACTAM not exceeding 2% w/v prepared with Sodium Chloride Injection, USP 0.9% or Dextrose Injection, USP 5%, to which clindamycin phosphate, gentamicin

sulfate, tobramycin sulfate, or cefazolin sodium have been added at concentrations usually used clinically, are stable for up to 48 hours at room temperature or 7 days under refrigeration. Ampicillin sodium admixtures with aztreonam in Sodium Chloride Injection, USP 0.9% are stable for 24 hours at room temperature and 48 hours under refrigeration; stability in Dextrose Injection, USP 5% is 2 hours at room temperature and 8 hours under refrigeration.

Aztreonam-cloxacillin sodium and aztreonam-vancomycin hydrochloride admixtures are stable in Dianeal 137 (Peritoneal Dialysis Solution) with 4.25% Dextrose for up to 24 hours at room temperature.

Aztreonam is incompatible with nafcillin sodium, cephradine, and metronidazole.

Other admixtures are not recommended since compatibility data are not available.

Intravenous (IV) Solutions

For Bolus Injection: The contents of an AZACTAM (aztreonam for injection, USP) 15 mL capacity vial should be constituted with 6 mL to 10 mL Sterile Water for Injection, USP.

For Infusion: If the contents of a 15 mL capacity vial are to be transferred to an appropriate infusion solution, each gram of aztreonam should be initially constituted with at least 3 mL Sterile Water for Injection, USP. Further dilution may be obtained with one of the following intravenous infusion solutions:

Sodium Chloride Injection, USP, 0.9%
Ringer's Injection, USP
Lactated Ringer's Injection, USP
Dextrose Injection, USP, 5% or 10%
Dextrose and Sodium Chloride Injection, USP, 5%:0.9%, 5%:0.45% or 5%:0.2%
Sodium Lactate Injection, USP (M/6 Sodium Lactate)
Ionosol® B and 5% Dextrose
Isolyte® E
Isolyte® E with 5% Dextrose
Isolyte® M with 5% Dextrose
Normosol®-R
Normosol®-R and 5% Dextrose
Normosol®-M and 5% Dextrose
Mannitol Injection, USP, 5% or 10%
Lactated Ringer's and 5% Dextrose Injection

Plasma-Lyte M and 5% Dextrose
10% Travert Injection
10% Travert and Electrolyte No. 1 Injection
10% Travert and Electrolyte No. 2 Injection
10% Travert and Electrolyte No. 3 Injection

Intramuscular (IM) Solutions

The contents of an AZACTAM 15 mL capacity vial should be constituted with at least 3 mL of an appropriate diluent per gram aztreonam. The following diluents may be used:

Sterile Water for Injection, USP
Sterile Bacteriostatic Water for Injection, USP (with benzyl alcohol or with methyl- and propylparabens)
Sodium Chloride Injection, USP, 0.9%
Bacteriostatic Sodium Chloride Injection, USP (with benzyl alcohol)

Stability Of IV And IM Solutions

AZACTAM solutions for IV infusion at concentrations not exceeding 2% w/v must be used within 48 hours following constitution if kept at controlled room temperature (59°- 86° F/15°- 30° C) or within 7 days if refrigerated (36°- 46° F/2°- 8° C).

AZACTAM solutions at concentrations exceeding 2% w/v, except those prepared with Sterile Water for Injection, USP or Sodium Chloride Injection, USP, should be used promptly after preparation; the two excepted solutions must be used within 48 hours if stored at controlled room temperature or within 7 days if refrigerated.

Intravenous Administration

Bolus Injection: A bolus injection may be used to initiate therapy. The dose should be **slowly** injected directly into a vein, or the tubing of a suitable administration set, over a period of 3 to 5 minutes (see next paragraph regarding flushing of tubing).

Infusion: With any intermittent infusion of aztreonam and another drug with which it is not pharmaceutically compatible, the common delivery tube should be flushed before and after delivery of aztreonam with any appropriate infusion solution compatible with both drug solutions; the drugs should not be delivered simultaneously. Any AZACTAM (aztreonam for injection, USP) infusion should be completed within a 20- to 60-minute period. With use of a *Y-type administration set*, careful attention should be given to the calculated volume of aztreonam solution required so that the entire dose will be infused. A volume control administration set may be used to deliver an initial dilution of

AZACTAM (see **Preparation Of Parenteral Solutions: Intravenous (IV) Solutions:** *For Infusion*) into a compatible infusion solution during administration; in this case, the final dilution of aztreonam should provide a concentration not exceeding 2% w/v.

Intramuscular Administration

The dose should be given by deep injection into a large muscle mass (such as the upper outer quadrant of the gluteus maximus or lateral part of the thigh). Aztreonam is well tolerated and should not be admixed with any local anesthetic agent.

HOW SUPPLIED

AZACTAM® (aztreonam for injection, USP)

Single-dose 15 mL capacity vials:

1 g/vial:	Packages of 10	NDC 51479-041-15
2 g/vial:	Packages of 10	NDC 51479-042-15

Storage

Store original packages at room temperature; avoid excessive heat.

ALSO SUPPLIED AS:

AZACTAM® (aztreonam injection) in GALAXY plastic container (PL 2040) as a frozen, 50 mL single-dose intravenous solution as follows:

1 g aztreonam/50 mL container:	Packages of 24	NDC 51479-048-01
2 g aztreonam/50 mL container:	Packages of 24	NDC 51479-049-01

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Bristol-Myers Squibb Company

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AZACTAM®

(aztreonam injection)

in GALAXY Plastic Container (PL 2040)
for Intravenous Use

Rx only

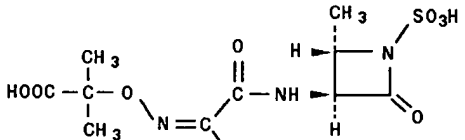
To reduce the development of drug-resistant bacteria and maintain the effectiveness of AZACTAM® and other antibacterial drugs, AZACTAM should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria.

DESCRIPTION

AZACTAM® (aztreonam injection) contains the active ingredient aztreonam, a monobactam. It was originally isolated from *Chromobacterium violaceum*. It is a synthetic bactericidal antibiotic.

The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics (eg, penicillins, cephalosporins, cephamycins). The sulfonic acid substituent in the 1-position of the ring activates the beta-lactam moiety; an aminothiazolyl oxime side chain in the 3-position and a methyl group in the 4-position confer the specific antibacterial spectrum and beta-lactamase stability.

Aztreonam is designated chemically as (Z)-2-[[[(2-amino-4-thiazolyl)[[(2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl]methylene]amino]oxy]-2-methylpropionic acid. Structural formula:



$C_{13}H_{17}N_5O_8S_2$ MW 435.44

AZACTAM (aztreonam injection) in the GALAXY plastic container (PL 2040) is a frozen, iso-osmotic, sterile, sodium-free, nonpyrogenic intravenous solution. Each 50 mL of solution contains 1 g, or 2 g aztreonam with approximately 1.7 g, or 700 mg dextrose hydrous, USP added to adjust osmolality, and approximately 780 mg, or 1.6 g of arginine added for pH adjustment, respectively. Thawed solutions have a pH in the range of 4.5 to 7.5. The solution is for intravenous administration following thawing at room temperature or under refrigeration.

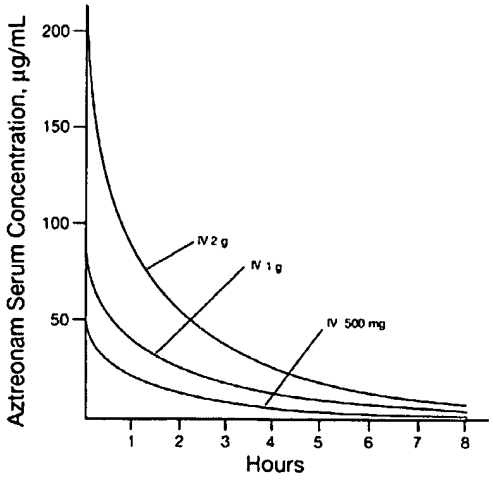
This GALAXY container is fabricated from a specially designed multilayer plastic (PL 2040). Solutions are in contact with the polyethylene layer of this container and can leach out certain chemical components of the plastic in very small amounts within the expiration period. The suitability of the plastic has been confirmed in tests in animals according to the USP biological tests for plastic containers as well as by tissue culture toxicity studies.

CLINICAL PHARMACOLOGY

Single 30-minute intravenous infusions of 500 mg, 1 g and 2 g doses of AZACTAM in healthy subjects produced aztreonam peak serum levels of 54 µg/mL, 90 µg/mL and 204 µg/mL, respectively, immediately after administration; at 8 hours, serum levels were 1 µg/mL, 3 µg/mL and 6 µg/mL, respectively (Figure 1). Single 3-minute intravenous injections of the same doses resulted in serum levels of 58 µg/mL, 125 µg/mL and 242 µg/mL at 5 minutes following completion of injection.

Serum concentrations of aztreonam following completion of single intravenous infusions of 500 mg, 1 g, and 2 g doses are depicted in Figure 1.

FIGURE 1



The serum levels of aztreonam following single 500 mg, 1 g or 2 g intravenous doses of AZACTAM (aztreonam injection) exceed the MIC_{90} for *Neisseria* sp., *Haemophilus influenzae* and most genera of the *Enterobacteriaceae* for 8 hours (for *Enterobacter* sp., the 8-hour serum levels exceed the MIC for

80% of strains). For *Pseudomonas aeruginosa,* a single 2 g intravenous dose produces serum levels that exceed the MIC_{90} for approximately 4 to 6 hours. All of the above doses of AZACTAM result in average urine levels of aztreonam that exceed the MIC_{90} for the same pathogens for up to 12 hours.

When aztreonam pharmacokinetics were assessed for adult and pediatric patients, they were found to be comparable (down to 9 months old). The serum half-life of aztreonam averaged 1.7 hours (1.5 to 2.0) in subjects with normal renal function, independent of the dose. In healthy subjects, based on a 70 kg person, the serum clearance was 91 mL/min and renal clearance was 56 mL/min; the apparent mean volume of distribution at steady-state averaged 12.6 liters, approximately equivalent to extracellular fluid volume.

In elderly patients, the mean serum half-life of aztreonam increased and the renal clearance decreased, consistent with the age-related decrease in creatinine clearance.[1-4] The dosage of AZACTAM should be adjusted accordingly (see **DOSAGE AND ADMINISTRATION: Renal Impairment in Adult Patients**).

In patients with impaired renal function, the serum half-life of aztreonam is prolonged. (See **DOSAGE AND ADMINISTRATION: Renal Impairment in Adult Patients.**) The serum half-life of aztreonam is only slightly prolonged in patients with hepatic impairment since the liver is a minor pathway of excretion.

Average urine concentrations of aztreonam were approximately 1100 µg/mL, 3500 µg/mL and 6600 µg/mL within the first 2 hours following single 500 mg, 1 g and 2 g intravenous doses of AZACTAM (30-minute infusions), respectively. The range of average concentrations for aztreonam in the 8- to 12-hour urine specimens in these studies was 25 µg/mL to 120 µg/mL. In healthy subjects, aztreonam is excreted in the urine about equally by active tubular secretion and glomerular filtration. Approximately 60% to 70% of an intravenous dose was recovered in the urine by 8 hours. Urinary excretion of a single intravenous dose was essentially complete by 12 hours after injection. About 12% of a single intravenous radiolabeled dose was recovered in the feces. Unchanged aztreonam and the inactive beta-lactam ring hydrolysis product of aztreonam were present in feces and urine.

Intravenous administration of a single 500 mg or 1 g dose of AZACTAM every 8 hours for 7 days to healthy subjects produced no apparent accumulation of aztreonam or modification of its disposition characteristics; serum protein binding averaged 56% and was independent of dose.

Renal function was monitored in healthy subjects given aztreonam; standard tests (serum creatinine, creatinine clearance, BUN, urinalysis and total urinary protein excretion) as well as special tests (excretion of N-acetyl-β-glucosaminidase, alanine aminopeptidase and β_2-microglobulin) were used. No abnormal results were obtained.

Aztreonam achieves measurable concentrations in the following body fluids and tissues:

EXTRAVASCULAR CONCENTRATIONS OF AZTREONAM AFTER A SINGLE INTRAVENOUS DOSE[1]

Fluid or Tissue	Dose (g)	Route	Hours Post-injection	Number of Patients	Mean Concentration (μg/mL or μg/g)
Fluids					
bile	1	IV	2	10	39
blister fluid	1	IV	1	6	20
bronchial secretion	2	IV	4	7	5
cerebrospinal fluid (inflamed meninges)	2	IV	0.9-4.3	16	3
pericardial fluid	2	IV	1	6	33
pleural fluid	2	IV	1.1-3.0	3	51
synovial fluid	2	IV	0.8-1.9	11	83
Tissues					
atrial appendage	2	IV	0.9-1.6	12	22
endometrium	2	IV	0.7-1.9	4	9
fallopian tube	2	IV	0.7-1.9	8	12
fat	2	IV	1.3-2.0	10	5
femur	2	IV	1.0-2.1	15	16
gallbladder	2	IV	0.8-1.3	4	23
kidney	2	IV	2.4-5.6	5	67
large intestine	2	IV	0.8-1.9	9	12
liver	2	IV	0.9-2.0	6	47
lung	2	IV	1.2-2.1	6	22
myometrium	2	IV	0.7-1.9	9	11
ovary	2	IV	0.7-1.9	7	13
skeletal muscle	2	IV	0.3-0.7	6	16
skin	2	IV	0.0-1.0	8	25
sternum	2	IV	1	6	6

[1]Tissue penetration is regarded as essential to therapeutic efficacy, but specific tissue levels have not been correlated with specific therapeutic effects.

The concentration of aztreonam in saliva at 30 minutes after a single 1 g intravenous dose (9 patients) was 0.2 μg/mL; in human milk at 2 hours after a single 1 g intravenous dose (6 patients), 0.2 μg/mL; in amniotic fluid at 6 to 8 hours after a single 1 g intravenous dose (5 patients), 2 μg/mL. The concentration of aztreonam in peritoneal fluid obtained 1 to 6 hours after multiple 2 g intravenous doses ranged between 12 μg/mL and 90 μg/mL in 7 of 8 patients studied.

Aztreonam given intravenously rapidly reaches therapeutic concentrations in peritoneal dialysis fluid; conversely, aztreonam given intraperitoneally in dialysis fluid rapidly produces therapeutic serum levels.

Concomitant administration of probenecid or furosemide and aztreonam causes clinically insignificant increases in the serum levels of aztreonam. Single-dose intravenous pharmacokinetic studies have not shown any significant interaction between aztreonam and concomitantly administered gentamicin, nafcillin sodium, cephradine, clindamycin or metronidazole. No reports of disulfiram-like reactions with alcohol ingestion have been noted; this is not unexpected since aztreonam does not contain a methyl-tetrazole side chain.

Microbiology

Aztreonam exhibits potent and specific activity *in vitro* against a wide spectrum of gram-negative aerobic pathogens including *Pseudomonas aeruginosa.* The bactericidal action of aztreonam results from the inhibition of bacterial cell wall synthesis due to a high affinity of aztreonam for penicillin binding protein 3 (PBP3). Aztreonam, unlike the majority of beta-lactam antibiotics, does not induce beta-lactamase activity and its molecular structure confers a high degree of resistance to hydrolysis by beta-lactamases (ie, penicillinases and cephalosporinases) produced by most gram-negative and gram-positive pathogens; it is, therefore, usually active against gram-negative aerobic microorganisms that are resistant to antibiotics hydrolyzed by beta-lactamases. It is active against many strains that are multiply-resistant to other antibiotics, such as certain cephalosporins, penicillin, and aminoglycosides. Aztreonam maintains its antimicrobial activity over a pH range of 6 to 8 *in vitro*, as well as in the presence of human serum and under anaerobic conditions.

Aztreonam has been shown to be active against most strains of the following microorganisms, both *in vitro* and in clinical infections as described in the **INDICATIONS AND USAGE** section.

Aerobic gram-negative microorganisms:

Citrobacter species, including *C. freundii*
Enterobacter species, including *E. cloacae*
Escherichia coli
Haemophilus influenzae (including ampicillin-resistant and other penicillinase-producing strains)
Klebsiella oxytoca
Klebsiella pneumoniae
Proteus mirabilis
Pseudomonas aeruginosa
Serratia species, including *S. marcescens*

The following *in vitro* data are available, **but their clinical significance is unknown**.

Aztreonam exhibits *in vitro* minimal inhibitory concentrations (MICs) of 8 µg/mL or less against most (≥90%) strains of the following microorganisms; however, the safety and effectiveness of aztreonam in treating clinical infections due to these microorganisms have not been established in adequate and well-controlled clinical trials.

Aerobic gram-negative microorganisms:

Aeromonas hydrophila
Morganella morganii
Neisseria gonorrhoeae (including penicillinase-producing strains)
Pasteurella multocida
Proteus vulgaris
Providencia stuartii
Providencia rettgeri
Yersinia enterocolitica

Aztreonam and aminoglycosides have been shown to be synergistic *in vitro* against most strains of *P. aeruginosa*, many strains of *Enterobacteriaceae*, and other gram-negative aerobic bacilli.

Alterations of the anaerobic intestinal flora by broad spectrum antibiotics may decrease colonization resistance, thus permitting overgrowth of potential pathogens, eg, *Candida* and *Clostridium* species. Aztreonam has little effect on the anaerobic intestinal microflora in *in vitro* studies. *Clostridium difficile* and its cytotoxin were not found in animal models following administration of aztreonam. (See **ADVERSE REACTIONS:** *Gastrointestinal.*)

Susceptibility Tests

Dilution Techniques: Quantitative methods are used to determine antimicrobial minimal inhibitory concentrations (MICs). These MICs provide estimates of the susceptibility of bacteria to antimicrobial compounds. The MICs should be determined using a standardized procedure. Standardized procedures are based on a dilution method[5] (broth or agar) or equivalent with standardized inoculum concentrations and standardized concentrations of aztreonam powder. The MIC values should be interpreted according to the following criteria:

For testing aerobic microorganisms other than *Haemophilus influenzae*:

MIC (µg/mL)	Interpretation
≤8	Susceptible (S)

16	Intermediate (I)
≥32	Resistant (R)

When testing *Haemophilus influenzae*[a]:

MIC (µg/mL)	Interpretation[b]
≤2	Susceptible (S)

a. Interpretative criteria applicable only to tests performed by broth microdilution method using *Haemophilus* Test Medium (HTM).[5]

b. The current absence of data on resistant strains precludes defining any categories other than "Susceptible." Strains yielding MIC results suggestive of a "nonsusceptible" category should be submitted to a reference laboratory for further testing.

A report of "Susceptible" indicates that the pathogen is likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable. A report of "Intermediate" indicates that the result should be considered equivocal, and, if the microorganism is not fully susceptible to alternative, clinically feasible drugs, the test should be repeated. This category implies possible clinical applicability in body sites where the drug is physiologically concentrated or in situations where high dosage of drug can be used. This category also provides a buffer zone which prevents small uncontrolled technical factors from causing major discrepancies in interpretation. A report of "Resistant" indicates that the pathogen is not likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable; other therapy should be selected.

Standardized susceptibility test procedures require the use of laboratory control microorganisms to control the technical aspects of the laboratory procedures. Standard aztreonam powder should provide the following MIC values:

Microorganism	MIC (µg/mL)
Escherichia coli ATCC 25922	0.06-0.25
Haemophilus influenzae[a] ATCC 49247	0.12-0.5
Pseudomonas aeruginosa ATCC 27853	2.0-8.0

a. Range applicable only to tests performed by broth microdilution method using *Haemophilus* Test Medium (HTM).[5]

Diffusion Techniques: Quantitative methods that require measurement of zone diameters also provide reproducible estimates of the susceptibility of bacteria to antimicrobial compounds. One such standardized procedure[6] requires the use of standardized inoculum concentrations. This procedure uses paper disks impregnated with 30 µg aztreonam to test the susceptibility of microorganisms to aztreonam.

Reports from the laboratory providing results of the standard single-disk susceptibility test with a 30 µg aztreonam disk should be interpreted according to the following criteria:

For testing aerobic microorganisms other than *Haemophilus influenzae*:

Zone diameter (mm)	Interpretation
≥22	Susceptible (S)
16 - 21	Intermediate (I)
≤15	Resistant (R)

When testing *Haemophilus influenzae*[a]:

Zone diameter (mm)	Interpretation[b]
≥26	Susceptible (S)

a. Interpretative criteria applicable only to tests performed by disk diffusion method using *Haemophilus* Test Medium (HTM).[6]

b. The current absence of data on resistant strains precludes defining any categories other than "Susceptible." Strains yielding zone diameter results suggestive of a "nonsusceptible" category should be submitted to a reference laboratory for further testing.

Interpretation should be as stated above for results using dilution techniques. Interpretation involves correlation of the diameter obtained in the disk test with the MIC for aztreonam.

As with standardized dilution techniques, diffusion methods require the use of laboratory control microorganisms that are used to control the technical aspects of the laboratory procedures. For the diffusion technique, the 30 µg aztreonam disk should provide the following zone diameters in these laboratory test quality control strains.

Microorganism	Zone diameter (mm)
Escherichia coli ATCC 25922	28-36 mm
Haemophilus influenzae[a] ATCC 49247	30-38 mm
Pseudomonas aeruginosa ATCC 27853	23-29 mm

a. Range applicable only to tests performed by disk diffusion method using *Haemophilus* Test Medium (HTM).[6]

INDICATIONS AND USAGE

To reduce the development of drug-resistant bacteria and maintain the effectiveness of AZACTAM® and other antibacterial drugs, AZACTAM should be used only to treat or prevent infections that are proven or strongly suspected to be caused by susceptible bacteria. When culture and susceptibility information are available, they should be considered in selecting or modifying antibacterial therapy. In the absence of such data, local epidemiology and susceptibility patterns may contribute to the empiric selection of therapy.

AZACTAM (aztreonam injection) is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms:

Urinary Tract Infections (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella oxytoca**, *Citrobacter* species* and *Serratia marcescens**.

Lower Respiratory Tract Infections, including pneumonia and bronchitis caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Proteus mirabilis*, *Enterobacter* species and *Serratia marcescens**.

Septicemia caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis**, *Serratia marcescens** and *Enterobacter* species.

Skin and Skin-Structure Infections, including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli*, *Proteus mirabilis*, *Serratia marcescens*, *Enterobacter* species, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Citrobacter* species*.

Intra-abdominal Infections, including peritonitis caused by *Escherichia coli*, *Klebsiella* species including *K. pneumoniae*, *Enterobacter* species including *E. cloacae**, *Pseudomonas aeruginosa*, *Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**.

Gynecologic Infections, including endometritis and pelvic cellulitis caused by *Escherichia coli*, *Klebsiella pneumoniae**, *Enterobacter* species* including *E. cloacae** and *Proteus mirabilis**.

AZACTAM (aztreonam injection) is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy

Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organism(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected as etiologic agents, therapy should be initiated using an anti-anaerobic agent concurrently with AZACTAM (see **DOSAGE AND ADMINISTRATION**). Certain antibiotics (eg, cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and

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*Efficacy for this organism in this organ system was studied in fewer than 10 infections.

Pseudomonas species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing of the causative organism(s), appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS

This preparation is contraindicated in patients with known hypersensitivity to aztreonam or any other component in the formulation.

WARNINGS

Both animal and human data suggest that AZACTAM is rarely cross-reactive with other beta-lactam antibiotics and weakly immunogenic. Treatment with aztreonam can result in hypersensitivity reactions in patients with or without prior exposure. (See **CONTRAINDICATIONS**.)

Careful inquiry should be made to determine whether the patient has any history of hypersensitivity reactions to any allergens.

While cross-reactivity of aztreonam with other beta-lactam antibiotics is rare, this drug should be administered with caution to any patient with a history of hypersensitivity to beta-lactams (eg, penicillins, cephalosporins, and/or carbapenems). Treatment with aztreonam can result in hypersensitivity reactions in patients with or without prior exposure to aztreonam. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (eg, maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures. (See **ADVERSE REACTIONS**.)

Clostridium difficile associated diarrhea (CDAD) has been reported with use of nearly all antibacterial agents, including AZACTAM, and may range in severity from mild diarrhea to fatal colitis. Treatment with antibacterial agents alters the normal flora of the colon leading to overgrowth of *C. difficile*.

C. difficile produces toxins A and B which contribute to the development of CDAD. Hypertoxin-producing strains of *C. difficile* cause increased morbidity and mortality, as these infections can be refractory to antimicrobial therapy and may require colectomy. CDAD must be considered in all patients who present with diarrhea following antibiotic use. Careful medical history is necessary since CDAD has been reported to occur over two months after the administration of antibacterial agents.

If CDAD is suspected or confirmed, ongoing antibiotic use not directed against *C. difficile* may need to be discontinued. Appropriate fluid and electrolyte management, protein supplementation, antibiotic treatment of *C. difficile*, and surgical evaluation should be instituted as clinically indicated.

Rare cases of toxic epidermal necrolysis have been reported in association with aztreonam in patients undergoing bone marrow transplant with multiple risk factors including sepsis, radiation therapy and other concomitantly administered drugs associated with toxic epidermal necrolysis.

PRECAUTIONS

General

Prescribing AZACTAM in the absence of a proven or strongly suspected bacterial infection or a prophylactic indication is unlikely to provide benefit to the patient and increases the risk of the development of drug-resistant bacteria.

In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy.

If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics.

The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms (*Staphylococcus aureus* and *Streptococcus faecalis*) and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Information for Patients

Patients should be counseled that antibacterial drugs including AZACTAM should only be used to treat bacterial infections. They do not treat viral infections (eg, the common cold). When AZACTAM is prescribed to treat a bacterial infection, patients should be told that although it is common to feel better early in the course of therapy, the medication should be taken exactly as directed. Skipping doses or not completing the full course of therapy may (1) decrease the effectiveness of the immediate treatment and (2) increase the likelihood that bacteria will develop resistance and will not be treatable by AZACTAM or other antibacterial drugs in the future.

Diarrhea is a common problem caused by antibiotics which usually ends when the antibiotic is discontinued. Sometimes after starting treatment with antibiotics, patients can develop watery and bloody stools (with or without stomach cramps and fever) even as late as two or more months after having taken the last dose of the antibiotic. If this occurs, patients should contact their physician as soon as possible.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies in animals have not been performed.

Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level.

Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy

Pregnancy Category B

Aztreonam crosses the placenta and enters the fetal circulation.

Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal, or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation.

There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers

Aztreonam is excreted in human milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use

The safety and effectiveness of intravenous AZACTAM have been established in the age groups 9 months to 16 years. Use of AZACTAM in these age groups is supported by evidence from adequate and well-controlled studies of AZACTAM in adults with additional efficacy, safety, and pharmacokinetic data from noncomparative clinical studies in pediatric patients. Sufficient data are not available for pediatric patients under 9 months of age or for the following treatment indications/pathogens: septicemia and skin and skin-structure infections (where the skin infection is believed or known to be due to *H. influenzae* type b). In pediatric patients with cystic fibrosis, higher doses of AZACTAM may be warranted. (See **CLINICAL PHARMACOLOGY, DOSAGE AND ADMINISTRATION,** and **CLINICAL STUDIES.**)

Geriatric Use

Clinical studies of AZACTAM did not include sufficient numbers of subjects aged 65 years and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients.[7-10] In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

In elderly patients, the mean serum half-life of aztreonam increased and the renal clearance decreased, consistent with the age-related decrease in creatinine clearance.[1-4] Since aztreonam is known to be substantially excreted by the kidney, the risk of toxic reactions to this drug may be greater in patients with impaired renal function. Because elderly patients are more likely to have decreased renal function, renal function should be monitored and dosage adjustments made accordingly (see **DOSAGE AND ADMINISTRATION: Renal Impairment in Adult Patients** and **Dosage in the Elderly**).

AZACTAM contains no sodium.

ADVERSE REACTIONS

Local reactions (eg, phlebitis/thrombophlebitis; discomfort/swelling) following IV administration occurred at rates of approximately 1.9%.

Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1% to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity:

Hypersensitivity—anaphylaxis, angioedema, bronchospasm

Hematologic—pancytopenia, neutropenia, thrombocytopenia, anemia, eosinophilia, leukocytosis, thrombocytosis

Gastrointestinal—abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment. (See **WARNINGS**.)

Dermatologic—toxic epidermal necrolysis (see **WARNINGS**), purpura, erythema multiforme, exfoliative dermatitis, urticaria, petechiae, pruritus, diaphoresis

Cardiovascular—hypotension, transient ECG changes (ventricular bigeminy and PVC), flushing

Respiratory—wheezing, dyspnea, chest pain

Hepatobiliary—hepatitis, jaundice

Nervous System—seizure, confusion, vertigo, paresthesia, insomnia, dizziness

Musculoskeletal—muscular aches

Special Senses—tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing, nasal congestion, halitosis

Other—vaginal candidiasis, vaginitis, breast tenderness

Body as a Whole—weakness, headache, fever, malaise

Pediatric Adverse Reactions

Of the 612 pediatric patients who were treated with AZACTAM in clinical trials, less than 1% required discontinuation of therapy due to adverse events. The following systemic adverse events, regardless of drug relationship, occurred in at least 1% of treated patients in domestic clinical trials: rash (4.3%), diarrhea (1.4%), and fever (1.0%). These adverse events were comparable to those observed in adult clinical trials.

In 343 pediatric patients receiving intravenous therapy, the following local reactions were noted: pain (12%), erythema (2.9%), induration (0.9%), and phlebitis (2.1%). In the US patient population, pain occurred in 1.5% of patients, while each of the remaining three local reactions had an incidence of 0.5%.

The following laboratory adverse events, regardless of drug relationship, occurred in at least 1% of treated patients: increased eosinophils (6.3%), increased platelets (3.6%), neutropenia (3.2%), increased AST (3.8%), increased ALT (6.5%), and increased serum creatinine (5.8%).

In US pediatric clinical trials, neutropenia (absolute neutrophil count less than $1000/mm^3$) occurred in 11.3% of patients (8/71) younger than 2 years receiving 30 mg/kg q6h. AST and ALT elevations to greater than 3 times the upper limit of normal were noted in 15% to 20% of patients aged 2 years or above receiving 50 mg/kg q6h. The increased frequency of these reported laboratory adverse events may be due to either increased severity of illness treated or higher doses of AZACTAM administered.

Adverse Laboratory Changes

Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were:

Hepatic—elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above).

Hematologic—increases in prothrombin and partial thromboplastin times, positive Coombs' test.

Renal—increases in serum creatinine.

OVERDOSAGE

If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION

Dosage in Adult Patients

AZACTAM (aztreonam injection), an intravenous solution in GALAXY plastic containers (PL 2040), is intended for intravenous use only. Dosage should be determined by susceptibility of the causative organisms, severity and site of infection, and the condition of the patient.

The intravenous route is recommended for patients with bacterial septicemia, localized parenchymal abscess (eg, intra-abdominal abscess), peritonitis or other severe systemic or life-threatening infections.

The duration of therapy depends on the severity of infection. Generally, AZACTAM should be continued for at least 48 hours after the patient becomes asymptomatic or evidence of bacterial eradication has been obtained. Persistent infections may require treatment for several weeks. Doses smaller than those indicated should not be used.

Renal Impairment in Adult Patients

Prolonged serum levels of aztreonam may occur in patients with transient or persistent renal insufficiency. Therefore, the dosage of AZACTAM should be halved in patients with estimated creatinine clearances between 10 mL/min/1.73 m^2 and 30 mL/min/1.73 m^2 after an initial loading dose of 1 g or 2 g.

When only the serum creatinine concentration is available, the following formula (based on sex, weight, and age of the patient) may be used to approximate the creatinine clearance (Clcr). The serum creatinine should represent a steady state of renal function.

$$\text{Males: Clcr} = \frac{\text{weight (kg) x (140-age)}}{\text{72 x serum creatinine (mg/dL)}}$$

Females: 0.85 x above value

In patients with severe renal failure (creatinine clearance less than 10 mL/min/1.73 m^2), such as those supported by hemodialysis, the usual dose of 500 mg, 1 g or 2 g should be given initially. The maintenance dose should be one-fourth of the usual initial dose given at the usual fixed interval of 6, 8 or 12 hours. For serious or life-threatening infections, in addition to the maintenance doses, one-eighth of the initial dose should be given after each hemodialysis session.

Dosage in the Elderly

Renal status is a major determinant of dosage in the elderly; these patients in particular may have diminished renal function. Serum creatinine may not be an accurate determinant of renal status. Therefore, as with all antibiotics eliminated by the kidneys, estimates of creatinine clearance should be obtained, and appropriate dosage modifications made if necessary.

Dosage in Pediatric Patients

AZACTAM should be administered intravenously to pediatric patients with normal renal function. There are insufficient data regarding intramuscular administration to pediatric patients or dosing in pediatric patients with renal impairment. (See **PRECAUTIONS: Pediatric Use.**)

AZACTAM DOSAGE GUIDELINES

Type of Infection	Dose	Frequency (hours)
ADULTS*		
Urinary tract infections	500 mg or 1 g	8 or 12
Moderately severe systemic infections	1 g or 2 g	8 or 12
Severe systemic or life-threatening infections	2 g	6 or 8
*Maximum recommended dose is 8 g per day		
PEDIATRIC PATIENTS**		
Mild to moderate infections	30 mg/kg	8
Moderate to severe infections	30 mg/kg	6 or 8
**Maximum recommended dose is 120 mg/kg/day		

Because of the serious nature of infections due to *Pseudomonas aeruginosa*, dosage of 2 g every 6 or 8 hours is recommended, at least upon initiation of therapy, in systemic infections caused by this organism in adults.

CLINICAL STUDIES

A total of 612 pediatric patients aged 1 month to 12 years were enrolled in uncontrolled clinical trials of aztreonam in the treatment of serious gram-negative infections, including urinary tract, lower respiratory tract, skin and skin-structure, and intra-abdominal infections.

Directions for Use of AZACTAM (aztreonam injection) in GALAXY Plastic Container (PL 2040).

AZACTAM (aztreonam injection) in GALAXY plastic container (PL 2040) is to be administered as an intermittent intravenous infusion only.

Storage

Store in a freezer capable of maintaining a temperature of -20° C (-4° F).

Thawing of Plastic Containers

Thaw frozen container at room temperature, 25° C (77° F) or in a refrigerator, 2° to 8° C (36° to 46° F). After thawing is complete, invert the container to assure a well-mixed solution. **(DO NOT FORCE THAW BY IMMERSION IN WATER BATHS OR BY MICROWAVE IRRADIATION.)**

Check for minute leaks by squeezing container firmly. If leaks are detected, discard solution as sterility may be impaired.

The container should be visually inspected. Thawed solutions should not be used unless clear; solutions will be colorless to yellow. Components of the solution may precipitate in the frozen state and will dissolve upon reaching room temperature with little or no agitation. If after visual inspection the solution remains discolored, cloudy, or if an insoluble precipitate is noted or if any seals or outlet ports are not intact, the container should be discarded.

DO NOT ADD SUPPLEMENTARY MEDICATION.

The thawed solution in GALAXY plastic container (PL 2040) remains chemically stable for either 14 days under refrigeration (2° to 8° C/36° to 46° F) or for 48 hours at room temperature (25° C/77° F). **DO NOT REFREEZE THAWED ANTIBIOTICS.**

Preparation for Intravenous Administration (Use aseptic technique)

1. Suspend container(s) from eyelet support.
2. Remove protector from outlet port at bottom of container.
3. Attach administration set. Refer to complete directions accompanying set.

Additives or other medication should not be added to AZACTAM (aztreonam injection) in GALAXY plastic container (PL 2040) or infused simultaneously through the same intravenous line. If the same intravenous line is used for sequential infusion of several different drugs, it should be flushed before and after infusion of AZACTAM (aztreonam injection) in GALAXY plastic container (PL 2040) with an infusion solution compatible with AZACTAM (aztreonam injection) in GALAXY plastic container (PL 2040)* and any other drug(s) administered via this common line.

It is recommended that the intravenous administration apparatus be replaced at least once every 48 hours.

CAUTION: Do not use plastic containers in series connections. Such use could result in an embolism due to residual air being drawn from the primary container before administration of the fluid from the secondary container is complete.

Intravenous Administration

Infusion of AZACTAM (aztreonam injection) in GALAXY plastic container (PL 2040) should be completed within a 20- to 60-minute period. The plastic container is a single-dose unit; discard any unused portion remaining in the container.

*The following infusion solutions are compatible with AZACTAM (aztreonam injection) in GALAXY plastic container (PL 2040):

Sodium Chloride Injection, USP, 0.9%
Ringer's Injection, USP
Lactated Ringer's Injection, USP
Dextrose Injection, USP, 5% or 10%
Dextrose and Sodium Chloride Injection, USP, 5%:0.9%,
5%:0.45% or 5%:0.2%
Sodium Lactate Injection, USP (M/6 Sodium Lactate)
Ionosol® B and 5% Dextrose
Isolyte® E
Isolyte® E with 5% Dextrose
Isolyte® M with 5% Dextrose
Normosol®-R

Normosol®-R and 5% Dextrose
Normosol®-M and 5% Dextrose
Mannitol Injection, USP, 5% or 10%
Lactated Ringer's and 5% Dextrose Injection
Plasma-Lyte M and 5% Dextrose
10% Travert Injection
10% Travert and Electrolyte No. 1 Injection
10% Travert and Electrolyte No. 2 Injection
10% Travert and Electrolyte No. 3 Injection

HOW SUPPLIED

AZACTAM® (aztreonam injection) in GALAXY plastic container (PL 2040) is supplied as a frozen, 50 mL single-dose intravenous solution as follows:

1 g aztreonam/50 mL container:
Packages of 24 NDC 51479-048-01

2 g aztreonam/50 mL container:
Packages of 24 NDC 51479-049-01

Store at or below -20° C (-4° F) [See **Directions for Use of AZACTAM® (aztreonam injection) in GALAXY Plastic Container (PL 2040)**].

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AZACTAM® (aztreonam injection) in GALAXY plastic container (PL 2040) is
manufactured to Bristol-Myers Squibb
specifications by:
Baxter Healthcare Corporation
Deerfield, IL 60015
for:
Bristol-Myers Squibb Company
Princeton, NJ 08543
Distributed by:
Elan Pharmaceuticals, Inc.
San Diego, CA 92121 USA

1178463A1 / 6001714 Revised January 2007

EXHIBIT 5

US007214364B2

(12) **United States Patent**
Montgomery

(10) **Patent No.: US 7,214,364 B2**
(45) **Date of Patent: *May 8, 2007**

(54) **INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS**

(75) Inventor: **Alan Bruce Montgomery**, Medina, WA (US)

(73) Assignee: **Corus Pharma, Inc.**, Seattle, WA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 213 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **10/613,639**

(22) Filed: **Jul. 3, 2003**

(65) **Prior Publication Data**

US 2004/0062721 A1 Apr. 1, 2004

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/027,113, filed on Dec. 20, 2001, now Pat. No. 6,660,249.

(60) Provisional application No. 60/258,423, filed on Dec. 27, 2000.

(51) **Int. Cl.**

A61K 9/14	(2006.01)
A61K 31/15	(2006.01)
A61K 31/16	(2006.01)
A01K 31/16	(2006.01)
C07D 205/08	(2006.01)
C07D 205/09	(2006.01)
A01N 41/06	(2006.01)

(52) **U.S. Cl.** **424/46**; 514/210.03; 514/210.15; 514/837; 514/924; 514/932; 514/933; 514/951; 540/200; 540/355; 424/489

(58) **Field of Classification Search** 514/210.03, 514/210.15, 924, 932, 933, 951, 837; 424/46, 424/489; 540/200, 355

See application file for complete search history.

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(Continued)

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(57) **ABSTRACT**

A method and a composition for treatment of pulmonary bacterial infections caused by gram-negative bacteria suitable for treatment of infection caused by *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species, *Serratia marcescens* as well as those caused by *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa,* using a concentrated formulation of aztreonam lysinate delivered as an aerosol or dry powder formulation.

16 Claims, 3 Drawing Sheets

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Michael J. James, et al., Stability of Intravenous Admixtures of Aztreonam and Ampicillin, *American Journal of Hospital Pharmacy*, 42:1095-1100 (May 1985).

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FIG. 1A FIG. 1B

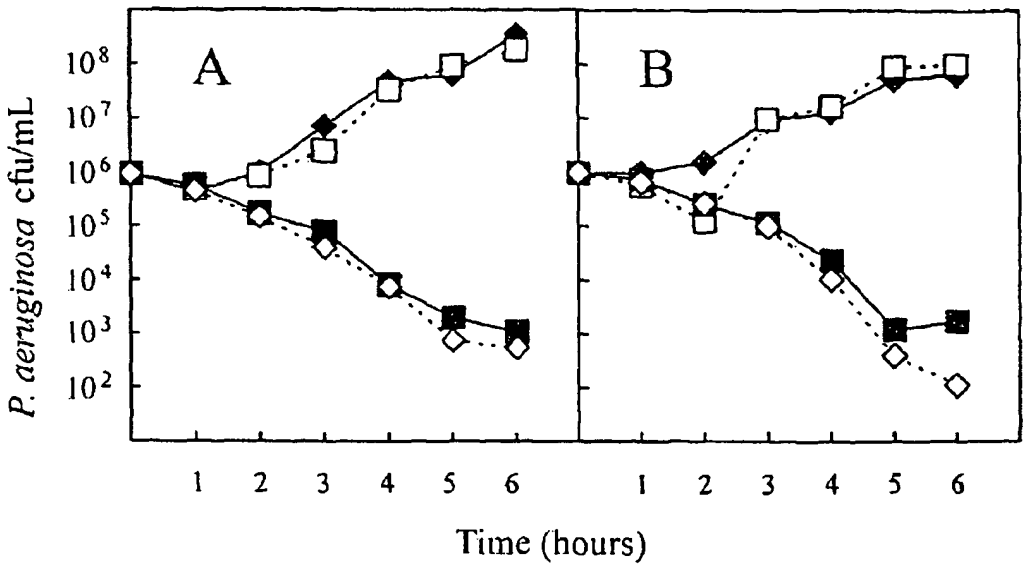
A
B
10^8
10^7
10^6
10^5
10^4
10^3
10^2
P. aeruginosa cfu/mL
1 2 3 4 5 6
1 2 3 4 5 6
Time (hours)

FIG. 2A

FIG. 2B

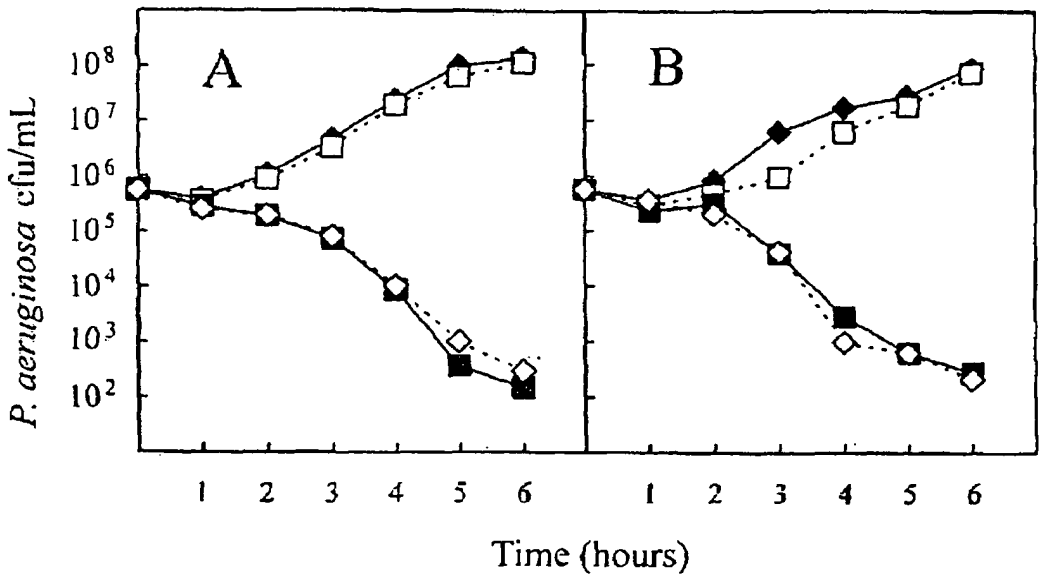
A
B
10^8
10^7
10^6
10^5
10^4
10^3
10^2
P. aeruginosa cfu/mL
1 2 3 4 5 6
1 2 3 4 5 6
Time (hours)

FIG. 3A

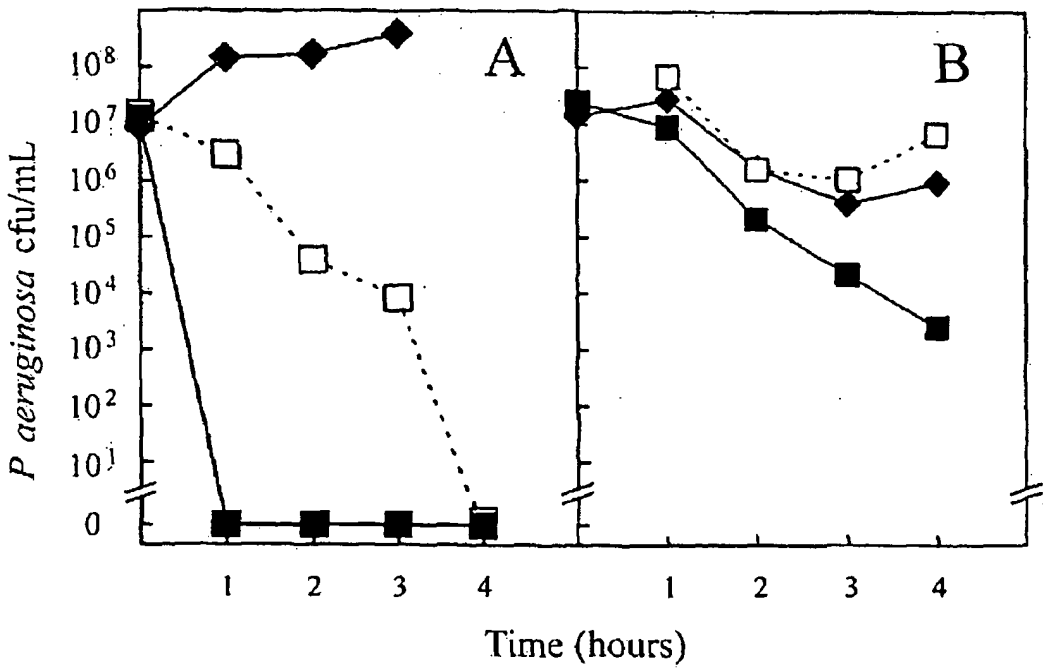
A
B
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
0
P aeruginosa cfu/mL
1
2
3
4
1
2
3
4
Time (hours)

FIG. 3B

INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS

This application is a continuation-in-part of U.S. application Ser. No.: 10/027,113 filed on Dec. 20, 2001 (now U.S. Pat. No. 6,660,249) which is based on and claims priority of the Provisional application Ser. No. 60/258,423, filed on Dec. 27, 2000.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The current invention concerns a novel, safe, nonirritating and physiologically compatible inhalable aztreonam lysinate formulation suitable for treatment of pulmonary bacterial infections caused by gram negative bacteria, such as *Escherichia coli, Enterobacteria* species, *Klebsiella pneumoniae, K. oxytoca, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Haemophilus influenzae, Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans.* In particular, the invention concerns the inhalable aztreonam lysinate formulation derived from aztreonam alpha form suitable for treatment and prophylaxis of acute and chronic pulmonary bacterial infections, particularly those caused by gram-negative bacteria *Burkholderia cepacia, Stenotrophomonas Maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa* which are resistant to treatment with other antibiotics.

The inhalable aztreonam lysinate formulation is delivered as an aerosol or as an inhalable dry powder. For aerosolization, about 1 to about 250 mg of aztreonam lysinate is dissolved in about 1 to about 5 ml of saline or other aqueous solution having a pH between 4.5 and 7.5, delivered to the lung endobronchial space in an aerosol having mass medium average diameter particles predominantly between 1 to 5μ using a nebulizer able to atomize the aztreonam lysinate solution into particles of required sizes. The aerosol formulation has a small volume yet delivers a therapeutically efficacious dose of aztreonam lysinate to the site of the infection in amounts sufficient to treat bacterial pulmonary infections. A combination of the novel formulation with the atomizing nebulizer permits about 50% delivery of the administered dose of aztreonam lysinate into airways. For delivery of dry inhalable powder, aztreonam lysinate is lyophilized, milled or spray dried to particle sizes between about 1 and 5μ. Both the dry powder formulation or a reconstituted aztreonam lysinate solid for aerosolization have a long shelf-life and storage stability.

2. Background and Related Disclosures

A wide variety of gram-negative bacteria cause severe pulmonary infections. Many of these bacteria are or become resistant to commonly used or specialty antibiotics and require treatment with new types of antibiotics. The pulmonary infections caused by gram-negative bacteria are particularly dangerous to patients who have decreased immunoprotective responses, such as, for example, cystic fibrosis and HIV patients, patients with bronchiectasis or those on mechanical ventilation.

Therefore, the bacterial respiratory infections caused by organisms resistant to antibiotics continues to be a major problem, particularly in immunocompromised or hospitalized patients, as well as in patients assisted by mechanical ventilation, as described in *Principles and Practice of Infectious Diseases,* Eds. Mandel, G. L., Bennett, J. E., and Dolin, R., Churchill Livingstone Inc., New York, N.Y., (1995).

Currently accepted therapy for severe bacterial respiratory tract infections, particularly for treatment of pneumonia in patients with underlying illnesses, includes treatment with various intravenous antibacterial agents, often used in two or three way combination. Most of these agents are not suitable, available or FDA approved for either oral or aerosol dosing. In some cases the efficacious systemic intravenous or oral dose, if oral delivery is possible, requires doses which are borderline or outright toxic thus often preventing a use of perfectly good antibiotic for treatment of the pulmonary infections.

Thus it would be desirable to have available other modes of delivery routes of these antibiotics enabling a targeted delivery of smaller amounts of the antibiotic to endobronchial space of airways for treatment of these bacterial infections rather than administering the antibiotic systemically in large amounts.

Additionally, chronically ill patients are often affected with infections caused by bacteria which are largely resistant to commonly used antibiotics or, upon extended use of certain antibiotic, often develop strong resistance to such antibiotic. For example, chronic pulmonary colonization with *Pseudomonas aeruginosa* in patients with cystic fibrosis is a principal cause of their high mortality. When established, the chronic pulmonary infection is very difficult, if not impossible, to eradicate. More than 60% of cystic fibrosis patients are colonized with *Pseudomonas aeruginosa* bacterium strains which are largely resistant to regular and specialty antibiotics, such as piperacillin, ticarcillin, meropenem, netilmicin and only little sensitive to azlocillin, ciprofloxacin, timentin and ceftazidime. Many strains have also been shown to develop resistance to tobramycin and to colistin, if used continuously.

Often, after prolonged antibiotic therapy, a superinfection with organisms intrinsically resistant to oral, intravenous or inhaled antibiotics develops in patients with cystic fibrosis and other chronic pulmonary infections. The four most common drug resistant organisms are *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa.*

Cystic fibrosis patients infected with *Burkholderia cepacia* have an increased rate of mortality compared to those patients with *Pseudomonas aeruginosa* infections. In some cystic fibrosis patients, *Burkholderia cepacia* can cause a rapid fatality, as described, for example in *Am. J. Respir. Crit. Care Med.,* 160: 5, 1572–7 (1999).

The high level of antibiotic resistance demonstrated by most strains of *Burkholderia cepacia* severely limits therapeutic options for its treatment (*Clinics Chest Med.,* 19:473–86 (September 1998)). Furthermore, unlike *Pseudomonas aeruginosa, Burkholderia cepacia* can cause epidemic spread among cystic fibrosis patients and therefore any patient infected with *Burkholderia cepacia* is usually isolated from other patients. This causes both additional expenses connected with caring for these patients and may also be psychologically devastating to the patient. Furthermore, most lung transplant centers will not perform a lung transplant on patients infected with *Burkholderia cepacia* (*Clinics Chest Med.,* 19:473–86 (September 1998)). Therefore, the *Burkholderia cepacia* infection is often viewed as a death sentence by patients with cystic fibrosis.

Burkholderia cepacia is usually resistant to the parenteral delivery of various antibiotics, including aztreonam lysinate, with showing only 5% of isolates to be sensitive to such treatment (*Antimicrob. Agents Chemother.,* 34: 3, 487–8

(March 1990)). Thus it would be advantageous to have available treatment for *Burkholderia cepacia* infections.

Other gram-negative bacteria intrinsically resistant to tobramycin can also complicate the care of a cystic fibrosis patient. These bacteria include *Stenotrophomonas maltophilia* and *Alcaligenes xylosoxidans*. Antibiotic therapy of these infections is usually also ineffective or leads to rapid emergence of drug resistance. Therefore, the successful treatment of all these infections requires that samples of these isolates are sent to a laboratory for complex antibiotic synergy determination of proper therapy for each individual patient (*Ped. Pulmon.*, S17: 118–119 (1998)). It would, therefore, be also advantageous to provide a therapy for these rare but hard to treat bacterial infections.

Similarly, the development of *P. aeruginosa* infection with strains which are resistant to, that is which have a high minimal inhibitory concentration (MIC) to a majority of antibiotics including tobramycin, predicts declining lung function and also may disqualify the patient from consideration for lung transplant (*Clinics Chest Med.*, 19:535–554 (September 1998)).

Existing antibiotic treatments for *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa* pulmonary infections are either ineffective, or lead to rapid emergence of drug resistance.

From the brief description above, it is clear that there is a continuous need for an effective therapy for treatment of acute and chronic pulmonary bacterial infections caused by gram-negative bacteria and particularly those caused by *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa* lung infections. Such therapy would preferably comprise an inhalation of the aerosolized drug formulation delivering a therapeutically effective amount of the drug directly to the endobronchial space of airways to avoid systemic treatment.

The problems connected with infections caused with these antibiotic resistant bacteria are very serious and it would be advantageous to have available more efficient modes of treatments with different types of antibiotics.

Aztreonam is a synthetic antibiotic which has a good biological activity against gram-negative bacteria and its arginine salt derived from the beta form has previously been used for intravenous treatment of bacterial infections. However, its use is severely limited due to its low efficacy requiring administration of very large intravenous doses between 1000 and 4000 mg a day in order to treat the infections caused by gram-negative bacteria and also by its salt derivatization which is not suitable for inhalation purposes. Although it would be an antibiotic of choice for complementary treatment of patients treated with tobramycin or other antibiotics, particularly in cystic fibrosis patients, such treatment is not practical because of the high doses required and because of the complication encountered with the arginine salt.

Aztreonam is currently only available as an arginine salt. Arginine has been shown to be toxic to the lung and causes lung tissue irritation, inflammation, bronchospasm and cough and therefore is not suitable for a delivery by aerosolization. Consequently, aztreonam arginine salt is not approved for inhalation use in the United States or elsewhere. However, as the antibiotic for treatment of pulmonary bacterial infections caused by gram negative bacteria, aztreonam could become a drug of choice for such treatment, if it could be delivered by inhalation in therapeutically effective concentrations directly to the lungs and if the problems connected with the aztreonam arginine could be overcome by providing a different, safer and physiologically acceptable salt derivative.

The efficacious administration of aztreonam by inhalation is further complicated by a lack of safe, physiologically acceptable and stable formulations for use by inhalation. Aside from the physiologically acceptable salt, such formulation must meet several criteria, such as certain size range of inhalable particles, certain pH range and certain degree of salinity. When the aerosol contains a large number of particles with a mass medium average diameter (MMAD) larger than 5μ, these are deposited in the upper airways decreasing the amount of antibiotic delivered to the site of infection in the endobronchial space of airways. Similarly, both highly acidic and alkaline or hypotonic or hypertonic conditions lead to respiratory complications, such as bronchospasm and cough, preventing inhalation of the drug.

Thus it would be advantageous and desirable to provide an inhalable formulation for delivery of aztreonam by aerosol or a dry powder formulation for treatment of pulmonary gram-negative bacterial infections and particularly those caused by drug resistant strains *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa,* wherein the formulation comprises a smallest possible therapeutically effective amount of drug in a form which does not cause pulmonary inflammation, wherein the pH is adjusted to physiologically acceptable levels, wherein the aqueous solution is isotonic and wherein said formulation has adequate shelf life suitable for commercial distribution, storage and use.

It is, therefore, a primary object of this invention to provide an inhalation aztreonam formulation suitable to efficacious delivery of aztreonam into lung for treatment of pulmonary gram-negative infections, especially those caused by *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa* by providing a safe, physiologically acceptable and efficacious formulation for inhalation using a pure concentrated aztreonam lysinate salt, which formulation contains sufficient but not excessive concentration of the aztreonam lysinate, which formulation can be efficiently aerosolized by nebulization using jet, ultrasonic or atomization nebulizers, into an aerosol having particle sizes within a range from 1 to 5μ, or administered as a dry powder, both well tolerated by cystic fibrosis patients and by patients with impaired pulmonary function due to infections, inflammation or another underlying disease.

All patents, patent applications and publications cited herein are hereby incorporated by reference.

SUMMARY

One aspect of this invention is a method for treatment of pulmonary infections caused by gram-negative bacteria by inhalation of aerosolized aztreonam lysinate.

Another aspect of this invention is a method for treatment of pulmonary bacterial infections caused by gram-negative bacteria, said method comprising administration of an inhalable concentrated pure aztreonam lysinate in a dry powder form or as an aerosol containing from about 1 to about 250 mg of aztreonam lysinate, said aztreonam lysinate administered in an inhalable dry powder form or dissolved in from about 1 to about 5 ml of an aerosolable solution of pH between 4.5 and 7.5 containing from about 0.1 to about 0.9% of chloride or other anion to the lung endobronchial space of airways of a patient in need thereof by nebulization in an

aerosol having a mass medium average diameter between about 1 and about 5μ, once, twice, three times or four times a day typically up to a daily dose aztreonam lysinate of 500 mg a day but in no instance more than 750 mg a day.

Yet another aspect of this invention is a method for treatment of pulmonary bacterial infections caused by *Escherichia coli, Enterobacteria* species, *Klebsiella pneumoniae, K. oxytoca, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Haemophilus influenzae, Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa* using an inhalable formulation of aztreonam lysinate delivered by inhalation to the endobronchial space of airways in a dry powder form or in an aerosol.

Another aspect of this invention is an inhalable pharmaceutically acceptable composition comprising from about 1 to about 250 mg, preferably about 10 to about 150, and most preferably 75 mg per one dose of aztreonam lysinate, said composition suitable for treatment of pulmonary bacterial infections caused by gram-negative bacteria wherein said aztreonam lysinate or the pharmaceutically acceptable salt thereof are prepared as an inhalable dry powder or as an aerosolable solution.

Still another aspect of this invention is an aerosolized aztreonam lysinate formulation comprising from about 25 to about 90 mg/mL, preferably 75 mg/ml of aztreonam lysinate dissolved in from about 1 to 5 ml of a normal or diluted saline or another aqueous solution, having pH between 4.2 and 7.5.

Still another aspect of the current invention is a formulation comprising from about 1 to about 250 mg of aztreonam lysinate in a diluted saline solution ranging from one tenth to a half normal saline or other aqueous solvent containing chloride or another anion, wherein said formulation has a pH between 5.5 and 7.0 and is delivered by aerosolization in about 1–5 ml of solution wherein aerosol has particles of the mass medium average diameter predominantly between 1 and 5μ, wherein said formulation is nebulized using a jet, atomizing, electronic or ultrasonic nebulizer.

Still yet another aspect of the current invention is a dry powder formulation comprising from about 1 to 200 mg of alpha form of aztreonam lysinate, wherein said formulation is lyophilized, milled, spray dried or precipitated into a fine powder having particles with the mass medium average diameter between 1 and 5μ used for inhalation of the dry powder administered from one to four times per day not exceeding 750 mg per day.

Another aspect of this invention is a two-part reconstitution system comprising an aztreonam lysinate in dry or lyophilized powder form and a diluent stored separately until use.

Still another aspect of this invention is a process for preparation of aztreonam lysinate from the alpha form of aztreonate wherein the resulting aztreonam lysinate has a better stability, higher purity and better yield.

BRIEF DESCRIPTION OF FIGURES

FIG. **1** shows aztreonam lysinate activity against *P. aeruginosa* in the absence (FIG. 1A) or presence (FIG. 1B) of hog gastric mucin. Aztreonam lysinate was added to yield a final concentration in the following multiples of the MIC: 0.0 (♦); 0.1 (□) ; 1.0 (■); and 10 (◇). FIG. 1A, no added mucin; FIG. 1B, 10% mucin added.

FIG. **2** shows aztreonam lysinate activity against *P. aeruginosa* in the presence or absence of cystic fibrosis (CF) sputum. Aztreonam lysinate was added to yield a final concentration in the following multiples of the MIC: 0.0 (♦); 0.1 (□); 1.0 (■); and 10 (◇). FIG. 2A, no added sputum; FIG. 2B, 1% sputum added.

FIG. **3** shows tobramycin activity against *P. aeruginosa* in the presence or absence of added mucin. Tobramycin was added to yield a final concentration in the following multiples of the MIC: 0.0 (♦); 1.0 (□); and 10 (■) FIG. 3A, no added mucin; FIG. 3B, 10% mucin added.

DEFINITIONS

As used herein:

"MMAD" means mass medium average diameter.

"Normal saline" means water solution containing 0.9% (w/v) NaCl.

"Diluted saline" means normal saline containing 0.9% (w/v) NaCl diluted into its lesser strength from about 0.1% to about 0.8%.

"Half normal saline" or "½ NS" means normal saline diluted to its half strength containing 0.45% (w/v) NaCl.

"Quarter normal saline" or "¼ NS" means normal saline diluted to its quarter strength containing 0.225% (w/v) NaCl.

"One tenth normal saline" or "1/10 NS" means normal saline diluted to its one tenth strength containing 0.09% (w/v) NaCl.

"CF" means cystic fibrosis.

"Predominantly" means including at least 70% but preferably 90% of particle sizes between 1 and 5μ.

"Physiologically acceptable solution" means a saline diluted to between 1/10 NS or 1 NS or another aqueous solution comprising from about 31 to about 154 mM of chloride or an equivalent concentration of bromine or iodine.

"Composition" means an aztreonam lysinate containing formulation additionally containing other components, such as excipients, diluents, isotonic solutions, buffers, etc.

"Formulation" means a specific composition formulated for specific use, such as for aerosolization of aztreonam lysinate containing solution or nebulization of dry powder.

"Aztreonam lysinate composition" or "aztreonam lysinate formulation" means a composition or formulation comprising an indicated amount of aztreonam lysinate salt. Thus, if for example, the dose of aztreonam lysinate comprises molar amount of aztreonam free base it contains 1.8 multiple molar amount of lysine.

"Concentrated aztreonam lysinate" means an aztreonam lysinate concentrated into a form which permits dilution of, or more than, 75 mg of aztreonam lysinate in 1 ml of diluent.

"Alpha form of aztreonam" means an alpha sterochemical configuration of aztreonam. The alpha form of aztreonam is distinguishable from the beta, gamma and delta forms of aztreonam. Each form seems to have different chemical and physical properties, such as, for example, stability, crystallization point and diffraction curve. Differences between these two forms are described, for example in U.S. Pat. No. 4,946,838. Alpha or beta aztreonam arginine salt are described in EP application 0 297 580 B1. Alpha, beta, gamma and delta forms of aztreonam and their chemical and physical properties are described in U.S. Pat. No. 4,826,973. All the above cited patents are herein incorporated by reference.

DETAILED DESCRIPTION OF THE INVENTION

The current invention concerns a discovery that a specifically formulated inhalable aztreonam lysinate is efficacious for treatment of pulmonary infections caused by gram-negative bacteria.

Consequently, the invention concerns an inhalable composition and a method of treatment for pulmonary bacterial infections caused by *Escherichia coli, Enterobacter* species, *Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Haemophilus influenzae,* including ampicillin-resistant and other penicillinases-producing strains and *Nitrobacter* species as well as for treatment of more rare bacteria, such as *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa.* The aztreonam lysinate formulation is delivered to a patient's endobronchial space of airways by inhalation of a dry powder or an aerosol solution.

The method of treatment of pulmonary bacterial infections is especially suitable for treatment of patients with cystic fibrosis, bronchiectasis and patients with pneumonia assisted by ventilators, however it is also useful for treatment of other conditions that are complicated by infections caused by *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa* or other gram-negative bacteria.

The current invention thus concerns a novel, efficacious, safe, nonirritating and physiologically compatible inhalable aztreonam lysinate composition suitable for treatment of pulmonary bacterial infections caused by gram-negative bacteria particularly those which are resistant to treatment with other antibiotics. The inhalable formulation of aztreonam lysinate is suitable both for treatment and prophylaxis of acute and chronic pulmonary infections. The inhalable formulation is delivered as an aerosol or as an inhalable dry powder. For aerosolization, aztreonam lysinate is dissolved in a minimal volume of about 1 to about 5 ml of an aqueous solvent comprising chloride bromine or iodine ion, having a pH between 4.2 and 7.5, delivered to the endobronchial space in an aerosol having mass medium average diameter particles predominantly between 1 to 5μ using a nebulizer able to aerosolize the aztreonam lysinate solution into particles of required sizes.

In another aspect, the current invention also concerns finding that the aztreonam lysinate derived from the alpha form of aztreonam, as compared to the beta form, has better properties and are more suited for preparation of aztreonam lysinate salt for inhalable product. The use of the alpha form for preparation of aztreonam lysinate provides demonstrable advantages in both manufacturing processes and results in the product with higher purity and better stability.

This aspect is novel in that until now, the alpha form of aztreonam was described as unstable and its conversion to beta form of aztreonam was required for preparation of therapeutic agents. The findings described herein are related to processes connected with formation of the aztreonam lysinate salt.

I. Aztreonam Generally

Aztreonam is a compound known under its chemical name (Z)-2-[[[(2-amino-4-thiazolyl)[[(2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl]methylene]amino]oxy]-2-methylpropionic acid.

Aztreonam is a known synthetic antibiotic with antibacterial activity against most gram-negative bacteria. Aztreonam is a monobactam and as such it has a unique monocyclic beta-lactam nucleus, and is therefore structurally different from other β-lactam antibiotics such as, for example penicillins, cephalosporins, or cephamycins. The sulfonic acid substituent in the 1-position of the ring activates the beta-lactam moiety. An aminothiazolyl oxime side chain in the 3-position and a methyl group in the 4-position confer the specific antibacterial spectrum and beta-lactamase stability.

Aztreonam is chemically known and available as alpha, beta, gamma and delta forms. Aztreonam arginine salt, known under its trade name AZACTAM® is derived from the beta form.

AZACTAM® (aztreonam arginine for injection, USP) commercially available from DURA Pharmaceuticals, Inc., San Diego, Calif., contains aztreonam as the active ingredient. AZACTAM is formulated as arginine salt and is currently FDA approved only for intramuscular or intravenous use (*PDR,* pg. 1159 (2001)).

A. Disadvantages of Aztreonam Arginine Salt

The commercially available AZACTAM for intravenous or intramuscular formulation is not suitable for inhalable use because of the presence of arginine in the formulation. Arginine has been found to cause pulmonary inflammation when administered in an aerosol form to the lung in the rat.

Arginine has been unsuccessfully used as a potential aerosolized mucolytic agent in cystic fibrosis patients. A study, described in *Pediatrics,* 55:96–100 (1975) recommends that arginine should not be used in cystic fibrosis patients. In a study of 24 patients with cystic fibrosis, inhalation therapy with an arginine solution in five patients had to be stopped because of the inflammation confirmed by bronchoscopy, cough and severe deterioration of their general conditions. Later, arginine was identified as a substrate for the production of nitric oxide radicals which are known to cause the lung inflammation, bronchospasm and irritation.

Nitric oxide radical reacts with the superoxide anion to form peronitrile, which is by itself toxic to the tissue and also may further react to form highly reactive and toxic hydroxyl radical. Since inflammation is a serious impairment for cystic fibrosis and all other diseases which this invention attempts to treat, use of arginine salt is not suitable as it would defeat this purpose and worsen rather than improve the patient conditions.

Arginine is also an important substrate for immune complex injury in the lung, as disclosed in *PNAS,* 14:6338–6342 (1991). Since the aerosolization concentrates high levels of the aerosolized drug in the lung as compared to dilution seen after intravenous administration, the aerosolization of the aztreonam arginine salt would be detrimental rather than advantageous for treatment of cystic fibrosis patients or patients suffering from pulmonary infections. Moreover, it would dilute and/or negate the effect of aztreonam.

Aztreonam, in any form, is not currently approved or used for inhalation treatment and aerosol administration in the United States. Consequently, there is no known aztreonam or aztreonam lysinate containing formulation available for aerosol delivery to the endobronchial space of airways.

The only attempt to deliver aztreonam arginine intermittently to cystic fibrosis subjects is described in *Spanish Annals on Pediatrics,* 40: No.3 (1994) where such delivery was made in an open label trial in cystic fibrosis patients with intermittently administered 500 and 1000 mg of AZACTAM USP arginine salt, twice a day for 21 days, using CR60 System 22 unit nebulizer. The intent of this study was to treat aztreonam sensitive *Pseudomonas aeruginosa* organisms, but not multidrug resistant *Pseudomonas aeruginosa.* No effort or speculation was to treat *Burkhold-*

eria cepacia, Stenotrophomonas maltophilia, infections caused by *Alcaligenes xylosoxidans* or other gram-negative bacteria.

In this study, the nebulized solution of aztreonam was delivered after the physical therapy session. Prior to the therapy session, the patients were administered 3 cc of saline alone or mixed with bronchodilators salbutamol or ipratropium bromide and fenoterol bromohidrate to prevent bronchospasm. The treatment described in this study thus required both the pretreatment with inhaled saline and/or bronchodilating agents and prior physical therapy session as well as administration of large doses of the drug to be administered twice a day. Although in about 80% of patients lung function has somehow improved, such improvement was not statistically significant. At least one patient could not tolerate the therapy due to bronchospasm. Most patients required administration of bronchodilators and all patients underwent physical therapy prior to aztreonam treatment in order to tolerate the administration of large doses of nebulized aztreonam. Aztreonam therapy was discontinued if in vitro resistance was found. One patient developed *Burkholderia cepacia,* which was viewed as superinfection, and a possible adverse outcome. The reference, although suggestive of efficacy in drug sensitive *Pseudomonas aeruginosa,* which is expected because the drug is known for its effect on the gram-negative bacteria, does not disclose the use of aztreonam, aztreonam lysinate, alpha form of aztreonam, its continuous use or the use of aztreonam or aztreonam lysinate for treatment of multidrug resistant *P. aeruginosa* and teaches away from use in *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa* infections. Furthermore, the high incidence of bronchospasm developed with use of the published formula requiring either discontinuation or pretreatment with bronchodilators indicates the need for a different formulation safe for inhalation use.

As discussed above, currently the only commercially available salt of aztreonam is arginine and, as also already discussed above, the aztreonam arginine salt is not suitable for inhalation administration because arginine, after aerosol exposure, is known to cause pulmonary inflammation, bronchospasm and cough. AZACTAM, aztreonam containing arginine salt, is not approved by regulatory authorities for inhalation use. Therefore, another aztreonam salt is needed to achieve a safe formulation of aztreonam for inhalation treatment of patients with pulmonary infections or those having impaired pulmonary function due to cystic fibrosis or bronchiectasis.

Since the aztreonam containing arginine is not suitable for inhalation according to this invention, other acid addition salts were prepared and tested. Aztreonam lysinate, particularly aztreonam lysinate derived from aztreonam alpha form, was found to be pharmacologically most acceptable for inhalation purposes when administered as a dry powder or aerosol without causing any undesirable reactions.

The preferred pharmaceutically acceptable aztreonam lysinate salt is derived from reaction of aztreonam or alpha aztreonam with lysine.

B. Alpha and Beta Aztreonam

Previously, a preparation of aztreonam arginine and other salts but no lysinate involved almost exclusively the beta form of aztreonam. Alpha form of aztreonam was previously thought to be unstable and unusable for preparation of therapeutic compositions. Beta form of aztreonam was considered to be the stable form and if the alpha form was used it was thought to be necessary to first convert the alpha form to the beta form of aztreonam.

The U.S. Pat. No. 4,946,838 presents conclusive evidence that the alpha form of aztreonam is unstable and before used for preparation of any therapeutic product it should be converted to the beta form of aztreonam. The EPO application EP 0 297 580 B1 describes preparation of aztreonam arginine salt derived from alpha or beta aztreonam. Other disclosed salts are sodium carbonate, sodium bicarbonate, sodium citrate, sodium phosphate and sodium hydroxide. The European Patent application thus discloses the use of amorphous, pharmaceutically acceptable aztreonam salts, specifically limited to arginine, sodium carbonate, sodium bicarbonate, sodium citrate, sodium phosphate, and sodium hydroxide. Aztreonam salt described therein is being prepared by lyophilization for parenteral use. Specifically, the application identifies alpha or beta form mixed with arginine or another salt in dry state and then mixed with water to bring the pH to 5.0. The application does not disclose the use of aztreonam for aerosol use or as the lysine salt.

Aztreonam can exist in anhydrous amorphous and crystalline forms and also in hydrated and solvated crystalline forms. The amorphous and hydrated forms interconvert under certain temperature and humidity conditions and are both unstable. The anhydrous crystalline and solvated forms show good stability and have not shown interconversion in the solid state. However in the presence of excipients that release moisture, the anhydrous crystalline form decomposes to an extent dependent on moisture content and temperature.

According to the prior art, the crystalline form of alpha form of aztreonam is considered to be unstable and must be converted to the beta form by recrystallization from ethanol. Following this recrystallization step, the beta form is considered to be very stable. However, the re-crystallized aztreonam contains 1–2% of residual organic solvent, typically ethanol.

Stability of the alpha or beta compound is determined by its loss at various temperatures. Thus, the prior art reports that after one week of storage alpha form experiences approximately 1% loss at room temperature and an 80% loss at 80° C. In contrast, the beta form, which after a 12 month storage at 5% to 75% relative humidity and at −20° C. to 40° C. was more stable. Under these range of conditions, the samples were found to have undergone slight increase (<2%) in impurity level by TLC method, and a drop of 3.0 to 3.5% in potency, as determined by HPLC.

In the process of developing this invention it was unexpectedly found that for preparation of a lyophilized form of aztreonam lysinate for aerosol an alpha form of aztreonam, previously thought to be unstable, was actually the preferred form for the starting material for the lysine salt conversion process.

When compared to the beta material, the alpha material was found to have fewer impurities. The type and degree of impurities in the inhalation formulation are important for and have specific impact on the long term stability of the drug and shelf-life of the final product. The beta form of aztreonam is manufactured from the alpha form using an ethanol re-crystallization process that results in 5000–10,000 ppm residual ethanol. USP for FDA limits is <5000 ppm. Over time, this presence of ethanol leads to the generation of an ethyl ester, an impurity, which is not present in the alpha form.

Additionally, the beta form of aztreonam is relatively insoluble in water and clumps during dissolution to make the lysine salt. This results in the formation of open-chain nucleophilic ring opening and results in an undesirable added impurity. Under the presence of moisture the open

chain can grow under various temperature and humidity conditions, leading to higher instability. Testing data shows the initial impurity levels generated from the beta form is in the 1% range, close to the FDA limit for the permissible impurity while the impurity levels of aztreonam lysinate generated from alpha form is less than 0.1%.

C. Aztreonam Lysinate

Aztreonam lysinate subject of this invention is derived preferentially from alpha aztreonam form, however, it can also be derived from other aztreonam forms. At this time, aztreonam lysinate, derived either from the alpha, beta, gamma or from another aztreonam form is not known and was never before described. The lysine salt of generic β-lactams but not aztreonam specifically is described in U.S. Pat. No. 4,550,105.

The production of aztreonam lysinate derived from alpha aztreonam form without converting the alpha form into the beta form is a novel process not disclosed or suggested by any prior art.

The current novel method for preparation of aztreonam for inhalation is based on the finding that the alpha form of aztreonam, when solubilized in water and stirred, forms an emulsion or smooth slurry and when a lysine salt solution is titrated to the mixture, results in a rapid formation of an amorphous lysine salt. This salt has similar stability characteristics to the lyophilized beta form, however, when the alpha derived lysinate is dried it does not cause the opening of the ring and thus the initial impurity levels generated from the alpha form is less than 0.1–1%, substantially less than FDA limit for the impurity.

Therefore, by using the alpha form of aztreonam, the final product contains much lower initial impurity levels, with higher stability and less degradation over time that leads to a product with a longer shelf life. In the current process for preparation aztreonam lysinate from alpha form, the basic salt conversion volumes, ratio of individual components and pH of the reaction mixture is titrated to a fixed level. Manufacturing of the product using the titration process of the invention confirms finding of less than 100 ppm of residual ethanol in the alpha form aztreonam lysinate compared to the beta form wherein the residual ethanol levels were up to 10,000 ppm in the same volume. By using the alpha form, the formation of ethyl ester, another impurity detected in the beta aztreonam forms is eliminated. Concerning the stability of the two formulations, the accelerated stability method shows that the beta form degrades from the initial 0.9% open chain to over 2% at 30 days whereas for alpha form an initial 0.06% open chain grows only to 1.2% after 90 days under the same testing conditions.

The prior art dealing with alpha and beta aztreonam involves conversion of the alpha form to the beta form. Such conversion step, if used for production of aztreonam lysinate necessarily involves combining the beta form of the aztreonam, having a pH of approximately 2.3, with the lysine component, having a pH of approximately 10, to yield the aztreonam lysinate as a final product. The addition of a lysine component to the beta form of the aztreonam creates excessive ion exchange in the titration of the aztreonam acid to a physiologically acceptable pH. Additionally, this reaction results in an undesirable side reaction with open chain formation of the beta lactam ring in the aztreonam further leading to the final product having a higher degree of impurity, instability and an undesirably high osmolality. Albeit, while the alpha form of aztreonam is preferred, the beta form aztreonam lysinate is also intended to be within the scope of this invention.

High osmolality is not a desirable property of the aztreonam for inhalation as will be described in greater detail below, as the inhalable aztreonam formulation requires very specific degree and range of osmolality (Section III. A4 and priority document Ser. No. 10/027,113). High osmolality may cause the patient to react to the inhalation with bronchospasm or cough.

Use of the alpha form of aztreonam and preparation of the lysinated salt using the current process produces a more stable product with a better pH profile, lower impurity content, longer stability and a desirably reduced osmolality.

Three potential techniques were developed to yield the aztreonam lysinate derived from the alpha form of aztreonam. All these techniques avoid conversion to the beta form. The first techniques involves titration of lysine salt into the alpha form of aztreonam. The second techniques involves vacuum-drying of the raw alpha aztreonam at the end point of the synthesis when the aztreonam is combined with lysine in a lyophilizer and the final aztreonam lysinate is produced directly. The third technique involves spray-drying of the alpha form of the aztreonam with lysine into a bulk solid, to produce the aztreonam lysinate as the final product without need of going through the conversion to the beta form.

As already discussed above, the use of the aztreonam beta form for production of aztreonam arginine requires an amount of ethanol solvent in quantities that cannot be readily removed. Such residual solvent leads to formation of an ethyl ester in the aztreonam product during the first few months of storage and leads to an impure final product having a lesser stability as well as the shorter shelf-life of the product.

The current preferred process for preparation of the aztreonam lysinate derived from alpha form thus comprises solubilization of alpha form of aztreonam in water and subsequent titration of an aqueous solution of solid form of lysine into the aztreonam to form the lysine salt. The mixture is then lyophilized or spray dried. The current process avoids cleavage of the beta lactam ring by advantageously employing a titration to achieve a desirable pH profile of the aztreonam lysinate which is contrary to the techniques used for beta aztreonam salt preparation which comprises combination of the dry powder of beta aztreonam with L-arginine in a mixture, followed by solubilization of the powder with water and titration to a final concentration.

In either of the techniques disclosed herein for preparation of the aztreonam lysinate derived from the alpha form of aztreonam, conversion to the beta form as well as all problems connected with production of the aztreonam derived from the beta form of aztreonam is avoided.

D. Aztreonam Lysinate Pharmacological Activity

Aztreonam lysinate exhibits potent and specific activity in vitro against a wide spectrum of gram-negative aerobic pathogens including *Pseudomonas aeruginosa*. The bactericidal action of aztreonam lysinate results from the inhibition of bacterial cell wall synthesis due to a high affinity of aztreonam lysinate for penicillin binding protein 3 (PBP3).

Aztreonam lysinate, unlike the majority of β-lactam antibiotics, does not induce β-lactamase activity and its molecular structure confers a high degree of resistance to hydrolysis by β-lactamases, such as penicillinases and cephalosporinases, produced by most gram-negative and gram-positive pathogens. Aztreonam lysinate is therefore especially effective against gram-negative aerobic organisms that are resistant to antibiotics hydrolyzed by β-lactamases.

Aztreonam lysinate maintains its antimicrobial activity at a pH ranging from 6 to 8 in vitro as well as in the presence

of human serum and under anaerobic conditions. Aztreonam lysinate is active in vitro and is effective in laboratory animal models and clinical infections against most strains of the following organisms, *Escherichia coli, Enterobacter* species, *Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Haemophilus influenzae,* and *Nitrobacter* species, including many that are multi-resistant to other antibiotics such as certain cephalosporins, penicillins, and aminoglycosides.

Currently, the only infections for which aztreonam arginine salt is FDA approved are those caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens.*

It has now been discovered that, all the above named bacterial strains as well as rare and highly resistant strains, such as *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa* are successfully eradicated by daily treatment with low doses between about 1 and about 250 mg, preferably about 75 mg/mL, of aztreonam lysinate, preferably administered once or twice a day, with total daily doses not exceeding 750 mg/day.

II. Aztreonam Lysinate Inhalable Composition

The current invention primarily concerns a concentrated inhalable aztreonam lysinate composition suitable for efficacious delivery of aztreonam lysinate into the endobronchial space of airways by aerosolization or as a dry powder.

The invention is most preferably suitable for formulation of concentrated aztreonam lysinate for aerosolization by atomizing, jet, ultrasonic, pressurized, vibrating porous plate or equivalent nebulizers or by dry powder inhalers which predominantly produce aztreonam lysinate aerosol or dry powder particles between 1 and 5μ. Such particle sizes are necessary for efficacious delivery of aztreonam lysinate into the endobronchial space to treat bacterial infections.

A. Aerosolized Aztreonam Lysinate Composition

Aztreonam lysinate composition for aerosolization is formulated for efficacious delivery of aerosolized aztreonam lysinate to the lung endobronchial space of airways.

The aerosol formulation is delivered in a total volume of between about 1 and about 5 ml of aqueous physiologically acceptable solution for one inhalation dose. When formulated and delivered according to the method of invention, it delivers a therapeutically efficacious dose of aztreonam lysinate to the site of the infection in amount sufficient to treat bacterial pulmonary infections.

A combination of the novel aqueous formulation with the atomizing, jet, pressurized, vibrating porous plate or ultrasonic nebulizer permits, depending on the nebulizer, about at least 20 to about 90%, typically about 70% delivery of the administered dose of aztreonam lysinate into airways.

The formulation contains a minimal yet efficacious amount of aztreonam lysinate from about 1 to about 250 mg, more preferably from about 25 to about 90 mg/mL, and most preferably about 75 mg/mL, formulated in the smallest possible volume of physiologically acceptable diluent having a certain degree of salinity and certain pH, adjusted to permit generation of an aztreonam lysinate aerosol well tolerated by patients but minimizing the development of secondary undesirable side effects such as bronchospasm and cough.

Primary requirements for aerosolized aztreonam lysinate formulation are its safety and efficacy. Additional advantages are lower cost, manufacturing convenience, purity of the product, practicality of use, long shelf-life, storage and manipulation of the aerosol device. These requirements for aerosolized aztreonam lysinate have now been found to be met by the formulation containing certain degree of salinity and have certain pH range.

A. Dosage of Aztreonam Lysinate

Aztreonam lysinate has a relatively short life-time. Its half life time is about 1–2 hours and within ten to twelve hours the whole aztreonam dose is eliminated. Consequently, the effective treatment of bacterial pulmonary infections requires a treatment regimen which provides sufficient amount of drug to maintain the antibacterial level of aztreonam lysinate in the lung. Such regimen thus requires administration of an inhalable aztreonam lysinate one to several, preferably two to four, times a day. Most preferred dosing regimen for patient convenience is once or twice a day, however, because of a specific effect aztreonam lysinate asserts on the bacteria, and because of its relatively short life-time of about 12 hours, more than twice a day dosing is often required for complete eradication of the bacteria from the endobronchial space.

It is therefore preferable to deliver aerosolized or dry powder aztreonam lysinate in a smallest therapeutically efficacious amount at least twice a day, in some instances three to four times, and exceptionally more than four times a day. A dose of aztreonam lysinate or a salt thereof is therefor set to be between 1 and 250 mg per one dose formulated in, most preferably, about 75 mg of aztreonam/mL.

Typically, one therapeutically effective dose contains between 1 and 250 mg, preferably between 25 to 90 mg of aztreonam lysinate, in equivalent, administered by means that provides at least about 50%–70% efficacy of aztreonam lysinate delivery to the endobronchial space. Thus, with about a 250 mg dose, 125 mg of aztreonam lysinate is delivered during each administration. 100–250 mg of aztreonam lysinate delivered to the lung has been found to be efficacious in eradication of bacteria. In no instance should one dose exceed 250 mg. Above this amount, aerosolization is difficult, the drug tends to precipitate, and larger volumes are necessary for its delivery by aerosol, which defeats the purpose of the invention to deliver the therapeutical amount of drug with the greatest efficiency.

Determination of effective dosage of administered aztreonam lysinate and the regimen used for treatment of each patient depends on the responsiveness of the individual patient to the treatment. The ultimate decisive factor is the expected level of aztreonam lysinate in the sputum after aerosolization. The optimal range of aztreonam lysinate in 1 ml of sputum at any given time should be in the 500 to 2000 μg/mL range. Thus, the frequency of the administration is correlated with the effectiveness of administered aztreonam lysinate.

The effectiveness of aerosolized aztreonam lysinate is surprisingly high when compared to effectiveness of the intravenously administered aztreonam lysinate where the serum peak levels following the maximum permitted dose 2,000 mg resulted only in 242 ug/mL of sputum. Following such intravenous administration, the 6 hours levels were found to be in the range of 16 ug/ml, which is the MIC for non-resistant *Pseudomonas aeruginosa.*

The new mode of administration permitting a noninvasive administration of small yet effective amounts of aztreonam lysinate directly into lungs is a great improvement compared to all previously known method used for delivery of aztreonam lysinate.

2. Effect of pH on Aztreonam Lysinate Formulation

The solution or diluent used for preparation of aztreonam lysinate aerosol has a limited pH range from 4.2 to 7.5, preferably between 5.5 and 7.0.

The pH of the formulation is an important feature for aerosolized aztreonam lysinate delivery. When the aerosol is either acidic or basic, it can cause bronchospasm and cough. Although the safe range of pH is relative and some patients may tolerate a mildly acidic aerosol, others, particularly those with cystic fibrosis or other underlying disease will experience bronchospasm. Any aerosol with a pH of less than 4.5 typically induces bronchospasm. Aerosols with a pH between 4.5 and 5.5 will cause bronchospasm occasionally. Testing with aztreonam lysinate aerosol discovered that an aerosblizable aztreonam lysinate formulation having a pH between 5.5 and 7.0 is well tolerated and safe. Any aerosol having pH greater than 7.5 is to be avoided as the body tissues are unable to buffer alkaline aerosols. Aerosol with controlled pH below 4.5 and over 7.5 causes lung irritation accompanied by severe bronchospasm, cough and inflammatory reactions.

For these reasons as well as for the avoidance of bronchospasm cough or inflammation in patients, the optimum pH for the aerosol formulation was determined to be between pH 5.5 to pH 7.0.

Consequently, the aztreonam lysinate aerosol formulation is adjusted to pH between 4.5 and 7.5 with preferred pH range from about 5.5 to 7.0. Most preferred pH range is from 5.5 to 6.5.

3. Effect of Salinity on the Aztreonam Lysinate Formulation

Patients suffering from acute or chronic endobronchial infections and particularly those with cystic fibrosis or bronchiectasis have increased sensitivity to various chemical agents and have high incidence of bronchospastic, asthmatic or cough incidents. Their airways are particularly sensitive to hypotonic or hypertonic and acidic or alkaline conditions and to the presence of any permanent ion, such as chloride. Any imbalance in these conditions or the absence of chloride below certain values leads to bronchospastic or inflammatory events and/or cough which greatly impair treatment with inhalable formulations. Both these conditions prevent efficient delivery of aerosolized aztreonam lysinate into the endobronchial space. The clinical manifestations of the irritated airways are extremely undesirable.

Clearly, for aztreonam lysinate, it is not possible to use solely an aqueous solvent without providing certain degree of osmolality to meet and emulate physiological conditions found in healthy lungs. Consequently, certain amount of the chloride or another anion is needed for successful and efficacious delivery of aerosolized aztreonam lysinate but such amount is much lower than amounts provided and typically used for aerosols of other compounds.

Bronchospasm or cough reflexes do not respond to the same osmolality of the diluent for aerosolization, however, they can be sufficiently controlled and/or suppressed when the osmolality of the diluent is in a certain range. Preferred solution for nebulization of aztreonam lysinate which is safe and has airways tolerance has a total osmolality between 50 and 550 mOsm/kg with a range of chloride concentration of between 31 mM and 300 mM. The given osmolality controls bronchospasm, the chloride concentration, as a permeant anion, controls cough. In this regard the chloride anion can be substituted with bromine or iodine anions, since both are permeant anions. In addition, bicarbonate may be wholly or partially substituted for chloride ion. Normal saline (NS) contains 154 mM of chloride whereas 31 mM of chloride corresponds to about 0.2 normal saline.

Consequently, the formulation for aztreonam lysinate aerosol of the invention comprises from about 1 to about 90 mg, preferably about 75 mg, of aztreonam lysinate dissolved in 1 ml of a normal, or preferably a diluted saline to from about 1/10 normal saline (NS) to about and at most to 1 NS solution, preferably from about 1/10 to about 1/4 NS, that is a one tenth to one quarter diluted normal saline. It has now been discovered that aztreonam lysinate is efficaciously delivered into lungs when dissolved in lesser than normal saline, that is saline containing 0.9% of sodium chloride, and that the concentration of a chloride ion equal to or lesser than 1/4 N saline permits and assures a delivery of aztreonam lysinate into endobronchial space.

The aztreonam lysinate formulation containing about 50 mg of aztreonam lysinate per 1 ml of 0.2 NS has an osmolality of about 290 mOsm/l. Such osmolality is within a safe range of aerosols suitable for administration to patients suffering from pulmonary bacterial infections and also those patients with a cystic fibrosis or bronchiectasis.

An additional feature and advantage of using 1/10 to 1/4 NS solution comprising 50 mg/ml aztreonam lysinate is that the resulting aerosol formulation is very efficiently nebulized by an atomic, jet or ultrasonic nebulizer compared to aztreonam lysinate dissolved in a normal saline. Since the delivery of aztreonam lysinate formulated as described herein is much more efficient, much lower amount of aztreonam lysinate is needed to achieve complete eradication of gram-negative bacteria in lungs. Instead of 1000 to 4000 mg of aztreonam which was shown to be somehow effective in the only one prior attempt to aerosolize aztreonam, the formulation of aztreonam lysinate according to this invention permits treatments with as little as 1 mg/ml and with at most up to 50 mg/ml of aztreonam lysinate in a maximum amount of 5 ml volume, delivered preferably with an atomizing, jet, electronic or ultrasonic nebulizer.

4. Aerosolizable Aztreonam Lysinate Formulation

The aztreonam lysinate aerosolizable formulation comprises from about 1 to about 250 mg, preferably formulated in about 25 to about 90 mg/ml, most preferably about 75 mg/mL of aztreonam lysinate dissolved in about 1 to 5 ml of an aqueous solution containing low concentration of chloride ion between 0.09% and 0.9%, having pH adjusted to between 4.2 and 7.5, said formulation delivered by aerosolization using an atomizing, jet, electronic, ultrasonic nebulizer.

The most preferred aerosol formulation for aztreonam lysinate comprises 75 mg/mL of aztreonam lysinate dissolved in about 1–5 ml of a saline diluted preferably to a quarter (0.225%) or one tenth (0.09%) strength of normal saline, having pH adjusted to between 5.5 and 7.0, delivered by nebulization in aerosol particles having the mass medium average diameter predominantly between 1 and 5μ, wherein said formulation is nebulized using an atomizing, jet, electronic or ultrasonic nebulizer. Dose of aztreonam is recalculated to refer only to an aztreonam component.

Using the PARI E-flow nebulizer commercially available from PARI, Starnberg Germany, the delivery time for one ml of 75 mg/mL aztreonam lysinate solution is 3 minutes compared to 4 minutes for the 90 mg/mL aztreonam lysinate solution. The delivery is 25 mg aztreonam per minute is faster for the 75 mg/mL than the delivery of 22.5 mg aztreonam per minute for the 90 mg/mL solution. Since time of delivery is important from a patient perspective and improves compliance, the discovery that 75 mg/mL formulation is delivered faster than the 90 mg/mL is important as

well as unexpected. The 90 mg/mL is the maximum concentration of aztreonam lysinate that can be dissolved in 1 ml of the solution.

It was further discovered that the highest dissolvable concentration, i.e. 90 mg/mL, is not as well nebulizable as the 75 mg/mL concentration. Upon further investigation, it was determined that this is likely due to the viscosity of the solutions at each concentration as follows

Concentration of aztreonam	Viscosity of aztreonam
75 mg/mL	1.48 ± 0.1 mPas
90 mg/mL	1.7 ± 0.03 mPas

These findings are counterintuitive and surprisingly show that the lower concentration of the drug, namely 75 mg/mL formulation is the best dose for the most efficacious delivery of aztreonam lysinate by inhalation.

5. Dry Powder, Aerosol and Aerbsol Suspensions

The formulation according to the invention contains aztreonam lysinate formulated as a dry powder, aerosol solution or aerosol suspension of liposomes or other microscopic particles in an aqueous solvent. The formulation is designed to be well tolerated and able to be reliably and completely nebulized to aerosol particles within the respirable size range of 1 to 5μ.

The doses are designed to contain as much as, but not more than, the necessary amount of a most active form of aztreonam lysinate to prevent colonization and/or to treat severe pulmonary infections caused by a range of susceptible gram-negative organisms.

Patients can be sensitive to pH, osmolality, and ionic content of a nebulized solution. Therefore these parameters are adjusted to be compatible with aztreonam lysinate chemistry and still tolerable to patients.

The formulation of the invention is nebulized predominantly into particle sizes allowing a delivery of the drug into the terminal and respiratory bronchioles where the bacteria reside during infection and in the larger airways during colonization.

For efficacious delivery of aztreonam lysinate to the lung endobronchial space of airways in an aerosol particle, the formation of an aerosol having a mass medium average diameter predominantly between 1 to 5μ is necessary. The formulated and delivered amount of aztreonam lysinate for treatment and prophylaxis of endobronchial bacterial infections must effectively target the lung surface. The formulation must have a smallest possible aerosolizable volume able to deliver an effective dose of aztreonam lysinate to the site of the infection. The formulation must additionally provide conditions which would not adversely affect the functionality of the airways. Consequently, the formulation must contain enough of the drug formulated under the conditions which allow its efficacious delivery while avoiding undesirable reactions. The new formulation according to the invention meets all these requirements.

One way to deliver inhalable aztreonam lysinate is by way of dry inhalable powder.

The aztreonam lysinate of the invention may be endobronchially administered in a dry powder formulation for efficacious delivery of the finely milled aztreonam powder into the endobronchial space using dry powder or metered dose inhalers as an alternative to aerosol delivery.

A dry powder formulation has potency, on a mass basis, which allows such alternative delivery of aztreonam lysinate as a dry powder using dry powder inhaler. A sufficiently potent formulation of aztreonam lysinate provides a dry powder which can be advantageously delivered by dry powder inhaler or by metered dose inhaler. For delivery of dry inhalable powder, aztreonam lysinate is milled, precipitated, spray dried or otherwise processed to particle sizes between about 1 and 5μ.

Dry powder formulation comprises from about 20 to 200 mg, preferably 10 to 100 mg of aztreonam lysinate.

For dry powder formulation of the invention, aztreonam lysinate is milled to a powder having mass median average diameters ranging from 1–5 microns by media milling, jet milling, spray drying or particle precipitation techniques as described in Example 6.

Briefly, for spray drying, aztreonam alpha form is suspended in water, stirred and cooled. L-Lysine dissolved in water is added slowly over about 3 to about 10 minutes, preferably about 6 minutes, until both components are almost completely dissolved. Solution is purified using a charcoal and filtered. Subsequently, the solution is spray dried using any suitable spay-drying equipment, such as, for example Buchi Mini Spray Dryer B-191.

Particle size determinations are made using a multi-stage Anderson cascade impactor or other suitable method. The Thermo Andersen Eight Stage Non-Viable Cascade Impactor is specifically cited within the US Pharmacopoeia Chapter 601 as a characterizing device for aerosols within metered-dose and dry powder inhalers. The Eight Stage Cascade Impactor utilizes eight jet stages enabling classification of aerosols from 9.0 micrometers to 0.4 micrometers (at 28.3 L/min) and allows airborne particulate to impact upon stainless steel impaction surfaces or a variety of filtration media substrates. A final filter collects all particles smaller than 0.4.

Media milling is accomplished by placing a drug substance into a mill containing, for example, stainless steel or ceramic balls and rotating or tumbling the material until the desired drug particle size ranges are achieved. Advantages of media milling include good size control, narrow product size ranges, high efficiencies of recovery, and readily scalable processes. Disadvantages include long manufacturing process times which takes from several hours to several days, the requirement that the milling media be separated from the product at completion, and the possibility of contamination of the product with the media.

Jet milling uses very high pressure air streams to collide particles with one another, with fine particles of the desired size being recovered from the mill. Advantages include rapidity of the manufacturing process and less energy transfer during milling, resulting in less temperature rise during the drug production. The jet milling process is completed in seconds to minutes. Disadvantages of the jet milling include poorer yield and collection efficiencies, with only 50 to 80% of recovery being a typical yield.

Spray-drying is another technique useful for preparation of inhalable dry powder. Spray drying involves spraying a fine mist of aztreonam lysinate solution onto a support and drying the particles. The particles are then collected. Spray drying has the advantage of being the least prone to degrading chemical entities. Adding a co-solvent which decreases the solubility of a drug to a uniform drug solution results in solution precipitation. When sufficient co-solvent is added, the solubility of the drug falls to the point where solid drug particles are formed which can be collected by filtration or centrifugation. Precipitation has the advantage of being highly reproducible, having a high yield of recovery and

being able to be performed under low temperature conditions, which reduce degradation.

Dry powder inhalation and metered dose inhalations are more practical when administered doses result in the delivery of at least about 10 mg, and more preferably about 25 to about 100 mg, of aztreonam lysinate to the lung of the patient receiving treatment. Depending on the efficiency of the dry powder delivery device, which is typically about 70%, typical effective dry powder dosage levels fall in the range of about 20 to about 60 mg of aztreonam lysinate. Therefore, typically more than one breath of drug is required.

In this aspect, the invention provides a sufficiently potent formulation of pure aztreonam lysinate in dry powder or metered dose form of drug particles milled or otherwise prepared to particle sizes predominantly with a range of 1 to 5 microns. Such formulation is practical and convenient because it does not require any further handling such as diluting the dry powder or filling an aerosol container. Further, it utilizes the devices that are sufficiently small, fully portable and do not require, for example, an air compressor which is needed for a jet nebulizer. Additionally, the dry powder formulation has a longer shelf life that the liquid aztreonam lysinate formulations for aerosolization. Aztreonam lysinate, when reconstituted into an aerosolizable solution, has only a limited shelf life at room temperature due to hydrolysis of the monobactam ring. Aztreonam lysinate dry powder does not have this problem.

The dry powder formulation is thus practical and convenient for ambulatory use because it does not require dilution or other handling, it has an extended shelf-life and storage stability and the dry powder inhalation delivery devices are portable and do not require an air compressor needed by aerosol nebulizers.

All techniques suitable for preparation of dry inhalable powders and any and all improvements thereof as well as any dry powder inhaler are intended to be within the scope of the invention.

B. Stability, Shelf-Life and Storage

Stability of the formulation is another very important issue for efficacious formulation. If the drug is degraded before aerosolization, a smaller amount of the drug is delivered to the lung thus impairing the treatment efficacy. Moreover, degradation of stored aztreonam lysinate may generate materials that are poorly tolerated by patients.

The dry form of aztreonam lysinate has at least 2 years long shelf life. The liquid forms of the aztreonam/arginine have a 24-hour stability at room temperature, 48 hours when refrigerated, and when frozen at −4° C., such stability can be extended to about three months. However, the stability of aztreonam arginine salt is an attribute of arginine. The stability of other salts, after liquid reconstitution may differ.

A long-term stability of aztreonam free base or aztreonam lysinate in aqueous solution may not provide a sufficiently long shelf life-which would be commercially acceptable. A liquid formulation, therefore, may require a separation of aztreonam lysinate from the appropriate diluent. For this reason, the formulation is preferably supplied in a dry form and can be a reconstituted prior to administration as described below.

A formulation for aerosolization is thus preferably provided as two separate components, one containing a dry aztreonam lysinate containing an appropriate diluent such as 0.1 to 0.9 N saline, bicarbonate or any equivalent acqueous solution, as described above. The formulation is reconstituted immediately prior to administration. This arrangement prevents problems connected with the long-term stability of aztreonam lysinate in aqueous solvents.

According to the invention, aztreonam lysinate for aerosolization is preferably formulated in a lyophilized dosage form intended for use as a dry powder for reconstitution before inhalation therapy. The formulation of aztreonam lysinate can be aseptically prepared as a lyophilized powder either for dry powder delivery or for reconstitution and delivery, or as a frozen solution, a liposomal suspension, or as microscopic particles. The storage suitability of the formulation allows reliable reconstitution of the formulated aztreonam lysinate suitable for aerosolization.

C. Formulation for Inhalation-Packaging

The formulation of the invention is packaged for delivery to a patient in a package comprising several components.

Exemplary formulation package, consists of two separately packaged components: the lyophilized aztreonam-lysine powder and the sterile saline diluent to reconstitute the powder prior to delivery by nebulization.

Each vial contains 90–110% of labeled amount of Aztreonam (75 mg) and Lysine (47 mg) as aztreonam lysinate. Aztreonam and lysine form an ionic salt, which readily dissolves in saline. The diluent is a sterile 1 mL vial of 0.17% Sodium Chloride Inhalation Solution (0.17 mg/mL NaCl). After reconstitution with 0.17% NaCl, the pH of the solution is 4.2–7.0 and the osmolality is from 350 to 500 mOsmol/kg. The aztreonam related impurities are the following: open-chain aztreonam, desulfonated aztreonam, aztreonam E-isomer, and t-Butyl-Aztreonam. The total impurities are less than 1%. Each known contaminant is less than <0.2%. Unknown impurities are less than <0.1%. All ingredients meet USP requirements with the exception of lysine monohydrate, which currently has no monograph in the USP. The formulation contains no preservatives.

III. Administration of Aztreonam Lysinate by Inhalation

Aztreonam lysinate is currently not available. The only available form of aztreonam is aztreonam arginine for parenteral use. Arginine is known to cause. pulmonary inflammation and irritation, as discussed above, and is, therefore, unsuitable for inhalation use.

A. Two Modes of Inhalable Administration

Administration of inhalable aztreonam lysinate is achieved either with aztreonam lysinate aerosol or with inhalable dry aztreonam lysinate powder.

An arginine free formulation according to the invention delivered by inhalation has been shown to safely treat respiratory infections caused by all susceptible gram-negative bacteria including *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *serratia marcescens,* as well as, and more importantly, antibiotics resistant strains *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa.*

B. Frequency of Dosing

Treatment of pulmonary infections caused by the above named bacteria is achieved by a treatment regimen which provides one to several, preferably one to two, times a day an inhalable aztreonam lysinate. Most preferred dosing regimen for patient covenience is once or twice a day, however, because of a specific effect aztreonam lysinate asserts on the bacteria, and because of its relatively short life-time of about 12 hours, more often dosing is sometimes required for complete eradication of the bacteria from the endobronchial space.

In patients with severely impaired lung function, the frequency of dosing may be increased up to about twelve

times a day each time, providing only such amount of aztreonam lysinate as necessary to maintain therapeutic level in the lung.

Aztreonam lysinate kills bacteria by lysing cell walls as long as the local concentration of antibiotic exceeds the bacteria minimal inhibitory concentration (*Med. Clinics N, Am.*, 79: 4, 733–743 (1995)). Because of the relatively rapid clearance of antibiotics from the respiratory tract due to mucociliary action, greater efficacy is obtained at a lower dose of administered aztreonam lysinate by treating a patient three, four or more times a day rather than administer the drug only once or twice. To this effect the aztreonam lysinate dose delivered by inhalation is at least four times and can be one thousand time lower then the aztreonam arginine dose delivered intravenously or utilized in the one attempt described above to deliver aztreonam arginine by aerosolization where 500–1000 mg was delivered twice a day to a total amount of 1000 mg for children under 5 years of age and 2000 mg for individuals older than 5 years.

The current daily doses of aztreonam lysinate can be as small as 2 mg. The typical upper limit is 500 mg of aztreonam lysinate per day delivered in two to four administrations. In extreme cases the dose may reach up to 750 mg per day delivered in three, four or more aerosol administrations. Typical and preferred range for one aerosol dosage is between 20 and 200 mg administered twice a day or between 10 and 100 mg administered three or four times per day. Most preferred dose is 75 mg/ml delivered twice or more times a day.

Aerosolization of aztreonam lysinate utilizes delivery of aerosolized aztreonam lysinate using atomizing, jet, ultrasonic, electronic or other equivalent nebulizers. Portable nebulizers, such as atomizing, ultrasonic and electronic nebulizers are preferred for ambulatory treatment. The jet nebulizers with a compressor nebulize the aztreonam lysinate formulation very efficiently but are more suitable for use in the hospital and doctor's office.

A dry powder inhalation, as the second mode of administration of the inhalable aztreonam lysinate utilizes the aztreonam lysinate dry powder formulation. Such formulation comprises a delivery of the finely milled aztreonam lysinate directly to the endobronchial space. In this instance, aztreonam lysinate is delivered into the endobronchial space using dry powder or metered dose inhalers. The aztreonam lysinate potency, determined on a mass basis, allows the inhalation of aztreonam lysinate powder, as an alternative mode of administration to the aerosol. Dry powder inhalation is most efficacious, practical and economical when administered doses contain less than 100 mg. The frequency of dosing, thus, is typically three or four times a day but also includes one or two or more than four times dosing regimen as this regimen depends on the need and condition of the patient.

The invention provides a sufficiently potent formulation of aztreonam lysinate in a form of dry powder delivered as metered dose inhalation of aztreonam lysinate particles milled or spray dried to particle sizes predominantly within a range of 1 to 5μ. Such dry powder delivery is possible and preferable particularly for ambulatory inhalation as it simplifies the delivery process. Such delivery is convenient because it does not require any further handling such as diluting the dry powder or mixing the powder with a solvent, etc. Further, the dry powder inhalation utilizes the devices that are sufficiently small, fully portable and do not require, for example, an air compressor which is needed for a jet nebulizer. Additionally, the dry powder formulation has even longer shelf life than the liquid aztreonam lysinate formulation for aerosolization.

The dosing regimen for both aerosol and dry powder aztreonam lysinate comprises from one to four, typically, or more than four times daily, in untypical cases, administration of the aerosol or dry powder.

Severely impaired cystic fibrosis patients, for example, may be able to withstand only one inhalation at a time but could repeat this inhalation of small amount of aztreonam lysinate every two, three or four hours to obtain sufficient level of aztreonam lysinate in the lungs.

IV. Devices for Delivery of Aerosolized Aztreonam Lysinate

A primary requirement of this invention is to deliver aztreonam lysinate efficiently to the endobronchial space of airways in a most economic way. Thus, the invention requires that at least 30–50%, preferably 70–90% of the active drug, that is aztreonam lysinate subjected to nebulization is in fact delivered to a site where it asserts its therapeutic effect.

A. Nebulizers

The composition of the invention described above provides the drug formulated in a solution permitting delivery of a therapeutically efficient amount of the drug, provided that the aerosol generated by the nebulization meets criteria required for such efficient delivery. The apparatus (nebulizer) which aerosolizes the formulation of aztreonam lysinate thus becomes a very important part of the invention.

There are quite a few nebulizer types currently commercially available. Not all of them are suitable for practicing this invention.

A nebulizer is selected primarily on the basis of allowing the formation of aztreonam lysinate aerosol having a mass medium average diameter predominantly between 1 to 5μ. The delivered amount of aztreonam lysinate must be efficacious for treatment and prophylaxis of endobronchial infections, particularly those caused by susceptible bacteria. The selected nebulizer thus must be able to efficiently aerosolize the formulation which has salinity, osmotic strength, and pH adjusted as to permit generation of aztreonam lysinate aerosol that is therapeutically effective and well tolerated by patients. The negulizer must be able to handle the formulation having a smallest possible aerosolizable volume and still able to deliver effective dose of aztreonam lysinate to the site of the infection. Additionally, the aerosolized formulation must not impair the functionality of the airways and must minimize undesirable side effects.

The inability of certain nebulizers to nebulize therapeutic quantities of drugs into small and uniform particle size aerosols is well known. For efficacious delivery of aztreonam lysinate a range of aerosolized particles with MMAD needed to deliver the drug to the endobronchial space, the site of the infection, is between 1–5μ. Many commercially available nebulizers are able to aerosolize large volumes of the solution with an aim to deliver at least 10% of the volume to the endobronchial space by producing around 90% of large aerosol particles above 5μ with a very large number of particles being in the range of 50–100μ. These nebulizers are inefficient and not suitable for delivery of aztreonam lysinate according to this invention.

In order to be therapeutically effective, the majority of aerosolized aztreonam lysinate particles should not have larger mass medium average diameter (MMAD) than between 1 and 5μ. When the aerosol contains a large number of particles with a MMAD larger than 5μ, these are depos-

ited in the upper airways decreasing the amount of antibiotic delivered to the site of infection in the lower respiratory tract.

Previously, two types of nebulizers, jet and ultrasonic, have been shown to be able to produce and deliver aerosol particles having sizes between 1 and 5μ. These particle size are optimal for treatment of pulmonary bacterial infection cause by gram-negative bacteria such as *Pseudomonas aeruginosa, Escherichia coli, Enterobacter* species, *Klebsiella pneumoniae, K. oxytoca, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Haemophilus influenzae, Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa*. However, unless a specially formulated solution is used, these nebulizers typically need larger volumes to administer sufficient amount of drug to obtain a therapeutic effect. Therefore, without a specially formulated aztreonam lysinate the efficient delivery of aztreonam lysinate is not achieved.

Nebulizer suitable for practicing this invention must be able to nebulize a small volume of the formulation efficiently, that is into the aerosol particle size predominantly in the range from 1–5μ. Predominantly in this application means that at least 70% but preferably more than 90% of all generated aerosol particles are within 1–5μ range.

Jet and ultrasonic nebulizers can produce and deliver particles between the 1 and 5μ particle size. A jet nebulizer utilizes air pressure breakage of an aqueous aztreonam lysinate solution into aerosol droplets. An ultrasonic nebulizer utilizes shearing of the aqueous aztreonam lysinate solution by a piezoelectric crystal.

Typically, however, the jet nebulizers are only about 10% efficient under clinical conditions, while the ultrasonic nebulizer are only about 5% efficient. The amount deposited and absorbed in the lungs is thus a fraction of the 10% in spite of the large amounts of the drug placed in the nebulizer.

One type of nebulizer which is suitable and preferred for aztreonam lysinate delivery is an atomizing nebulizer which consists of a liquid storage container in fluid contact with the diaphragm and inhalation and exhalation valves. For administration of the aztreonam lysinate formulation, 1 to 5 ml of the formulation is placed in the storage container, aerosol generator is engaged which produces atomized aerosol of particle sizes selectively between 1 and 5μ.

Typical nebulizing devices suitable for practicing this invention include atomizing nebulizers, or modified jet nebulizers, ultrasonic nebulizers, electronic nebulizers, vibrating porous plate nebulizers, and energized dry powder inhalers modified for handling small volume of highly concentrated drug in a specific formulation having a specific pH, osmolality and salinity. Most preferred nebulizer is the PARI inhalation nebulizer described in PCT/US00/29541 modified to meet the requirements of this invention.

B. Dry Powder Inhalers

Dry powder is administered as such using devices which deliver the dry powder directly to the lungs.

There are two major designs of dry powder inhalers. One design is the metering device in which a reservoir for the drug is placed within the device and the patient adds a dose of the drug into the inhalation chamber. The second is a factory-metered device in which each individual dose has been manufactured in a separate container. Both systems depend upon the formulation of drug into small particles of mass median diameters from 1 to 5 microns, and usually involve co-formulation with larger excipient particles (typically 100 micron diameter lactose particles). Drug powder is placed into the inhalation chamber (either by device metering or by breakage of a factory-metered dosage) and the inspiratory flow of the patient accelerates the powder out of the device and into the oral cavity. Non-laminar flow characteristics of the powder path cause the excipient-drug aggregates to decompose, and the mass of the large excipient particles causes their impaction at the back of the throat, while the smaller drug particles are deposited deep in the lungs.

Current technology for dry powder inhalers is such that payload limits are around 100 mg of powder. The lack of long-term stability of aztreonam lysinate in an aqueous solution due to hydrolysis allows dry powder inhaler technology to become a preferred delivery vehicle for aztreonam lysinate dry powder.

C. Aerosol or Dry Powder Particle Size

Particle size of the aztreonam lysinate aerosol formulation is one of the most important aspect of the invention. If the particle size is larger than 5μ then the particles are deposited in upper airways. If the particle size of the aerosol is smaller the 1μ then it does not get deposited in the endobronchial space but continues to be delivered into the alveoli and may get transferred into the systemic blood circulation.

A jet nebulizer utilizes air pressure to break a liquid solution into aerosol droplets. An ultrasonic nebulizer works by a piezoelectric crystal that shears a liquid into small aerosol droplets. A pressurized nebulization system forces solution under pressure through small pores to generate aerosol droplets. A vibrating porous plate device utilizes rapid vibration to shear a stream of liquid into appropriate droplet sizes. However, only some formulations of aztreonam lysinate can be efficiently nebulized as the devices are sensitive to pH and salinity.

In dry powder inhalers, the aztreonam lysinate dry powder prepared as described above in dosages from 1–100 mg, preferably from 10–50 mg of dry powder as particles having sizes between 1 and 5μ, is used directly.

D. Efficacy of Aztreonam Lysinate Nebulization

Selection and choice of the nebulizer greatly effects efficacy of the inhalable aztreonam lysinate delivery.

A combination of an aerosol formulation of aztreonam lysinate and a nebulizing device significantly enhance the efficiency and speed of drug administration. Currently, for example the average time for administration of other aerosolized drugs, such as for example tobramycin, is 15–20 minutes per dose. The time required for this treatment represents a significant burden to the patient and contribute to reduced compliance with the BID regimen.

Furthermore, the nebulizer system used for tobramycin administration is less efficient than new atomizing devices. The total deposited dose of tobramycin in the lung is in the 12 to 15% range. Approximately 30% of the dispensed drug remains in the nebulizer at the end of treatment, and of the portion that is aerosolized, about 30% is emitted as particles too large or small to reach the lower airways.

The novel atomizing nebulizer, with an output of 8 to 10 microliters/seconds, or 0.48 to 0.60 ml/minute, is capable of delivering drug material 2 to 4 times faster than the prior nebulizers exemplarized by PARI LC plus nebulizer. Furthermore, the novel nebulizer is able to aerosolize approximately 90% of the dispensed dose, with 85% or more of the aerosol particles being within the size range required for lower airway deposition. As a result, administration of a specifically designed formulation of aztreonam lysinate using the atomizing nebulizer leads to substantial improvement in local delivery to the airways, to a shorter time required for delivery and, depending on the final concentra-

tion of aztreonam lysinate solution, reduces treatment time to as little as three or four minutes.

V. Supporting Experimental Studies

Pseudomonas aeruginosa is the most common cause of chronic endobronchial infection in cystic fibrosis (CF) patients. This infection is a major cause of morbidity and mortality in these patients. Topical application of antibiotic agents inhaled as aerosol mists has demonstrated significant benefit to CF patients. Aerosolized antibiotic therapy with agents including carbenicillin, gentamicin, ticarcillin, tobramycin, and colistin but not aztreonam has been practiced for many years.

The most widely used aerosolized antibiotic for treatment of CF patients is tobramycin, which produces substantial improvements in pulmonary function and other clinical parameters. In vitro, tobramycin is active against most *P. aeruginosa* organisms in the absence of sputum; however, in the presence of sputum, tobramycin bioactivity is significantly reduced.

Aztreonam is a monobactam antibiotic with excellent activity against many aerobic gram-negative bacteria, including *P. aeruginosa*. It is currently approved as parenteral therapy for a variety of serious infections and has been widely used in control of pulmonary exacerbations in CF patients. Aztreonam has an antibacterial spectrum similar to the aminoglycoside antibiotics tobramycin and gentamicin. Its excellent activity against many aerobic gram-negative bacteria, including *P. aeruginosa*, has led to widespread use among CF patients, including intravenous administration as single agent therapy and in combination with other antibiotics for treatment of pulmonary exacerbations. These studies have demonstrated improvement in pulmonary function and clinical scores, as well as reductions in bacterial load and white blood cell counts. Additionally, aztreonam have been shown to have a potential for control of *Burkholderia cepacia*, a pathogen intrinsically resistant to the commonly used aminoglycoside antibiotics.

In order to determine whether aztreonam would be successful for treatment of *P. aeruginosa* and other bacterial infections, in the presence of sputum or mucin antagonized aztreonam bioactivity in vitro was investigated.

Experimental conditions are described in Example 8.

Results of these studies are described in FIGS. 1 to 3 which represent antibiotic killing curves obtained with different concentrations of the antibiotics aztreonam (FIGS. 1 and 2) and tobramycin (FIG. 3), in the presence or absence of mucin or CF sputum. Mucin is a model for the protein binding component of sputum.

FIG. 1 illustrates aztreonam activity against *P. aeruginosa* in the absence (FIG. 1A) or presence (FIG. 1B) of hog gastric mucin. Aztreonam was added to yield a final concentration in the following multiples of the MIC: 0.0 (♦); 0.1 (□); 1.0 (■); and 10 (◇).

As seen in FIG. 1, the curves without hog gastric mucin (FIG. 1A) and without hog gastric mucin (FIG. 1B) are virtually identical, indicating no measurable inhibition of the antibiotic by mucin.

FIG. 2 shows aztreonam activity against *P. aeruginosa* in the presence or absence of cystic fibrosis (CF) sputum. Aztreonam was added to yield a final concentration in the following multiples of the MIC: 0.0 (♦); 0.1 (□); 1.0 (■); and 10 (◇).

As seen in FIG. 2, the curves without CF sputum (FIG. 2A) and without sputum (FIG. 2B) are virtually identical, indicating no measurable inhibition of the antibiotic by CF sputum.

Tobramycin, which is known to bind mucins and to be inhibited by sputum and mucin, was tested with or without mucin in the same assay for comparative purposes.

FIG. 3 shows tobramycin activity against *P. aeruginosa* in the absence (FIG. 3A) or presence (FIG. 3B) of added mucin. Tobramycin was added to yield a final concentration in the following multiples of the MIC: 0.0 (♦); 1.0% (□); and 10% (■).

FIG. 3 demonstrates the ability of hog mucin to inhibit the activity of tobramycin. In the absence of mucin, tobramycin killed *P. aeruginosa* effectively, reducing colony counts by seven logs in one hour when applied at 10×MIC. In contrast, the same concentration of tobramycin in the presence of mucin caused much less killing: negligible amounts at one hour and only three to four logs at four hours. At 1×MIC, tobramycin killed seven logs of *P. aeruginosa* in four hours in the absence of mucin, but killed less than one log at four hours in the presence of mucin.

Neither CF sputum nor hog gastric mucin showed significant inhibition of the activity of aztreonam under the conditions of this assay. The *P. aeruginosa* killing curves obtained were virtually identical to controls lacking sputum or mucin. Growth of *P. aeruginosa* occurred, as expected, when aztreonam was added in quantities less than the MIC (upper curves in all figures), while effective killing occurred when aztreonam was present at or above the MIC (lower curves).

This contrasts with the result for tobramycin, an antibiotic known to be inhibited by CF sputum and hog gastric mucin. Addition of mucin to tobramycin resulted in decreased killing by up to four logs, depending on timing and the concentration of antibiotic used. These results confirm the validity of the mucin inhibition assay as a model for interpreting expected outcomes in the lungs of CF patients.

These results show that aztreonam is not inhibited by sputum of cystic fibrosis patients and that it will not be inhibited as a primary or a secondary complementary treatment when administered by inhalation, at least not to the extent that tobramycin is. This implies that aztreonam may be preferable to tobramycin in the treatment of respiratory infections in cystic fibrosis or other patients, as more antibiotic will be available to eradicate *Pseudomonas aeruginosa*.

VI. Treatment of Pulmonary Bacterial Infections

This invention provides an efficacious treatment and prevention of acute and chronic pulmonary bacterial infections caused by *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens*, as well as infection caused by antibiotic resistant strains *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans*, and multidrug resistant *Pseudomonas aeruginosa*.

A. Two Modes of Inhalable Treatment

A method for treatment of pulmonary infections comprises administration of aztreonam lysinate in inhalable form whether by aerosol or as a dry powder, several times a day. The aztreonam lysinate daily dose is between 1 and 500 mg/day, with exceptional dose up to 750 mg/day administered in from 1–50 mg/ml for aerosol and from 2 to 200 mg daily dose of dry powder administered in a dose of 1–100 mg/one treatment. The aztreonam lysinate dosage and dosing frequency depends on the type of bacterial infection, severity thereof, age of the patient, the conditions of the patient, etc. In case of cystic fibrosis patients where the lung air capacity is diminished, the dosing is more frequent with lower doses.

The dry powder formulation suitable for treatment of pulmonary infections comprises 1 to 200 mg, preferably about 10 to 100 mg, of powder in an amorphous or crystalline state in particle sizes between 1 and 5 microns in mass median average diameter necessary for efficacious delivery of aztreonam lysinate into the endobronchial space. The dry powder formulation is delivered one to four or more times daily, preferably twice daily. The dry powder formulation is temperature stable and has a physiologically acceptable pH of 4.2–7.5, preferably 5.5 to 7.0, and an over five year long shelf life.

B. Treatment of Infections in Patients with Suppurative Pulmonary Diseases

Aerosol therapy of this invention is particularly useful for treatment of patients suffering from suppurative pulmonary diseases and is especially suitable for treatment of patients with cystic fibrosis, bronchiectasis and those patients on the mechanical ventilation.

Previously, aerosol therapy for cystic fibrosis inhaled (ATCF) antibiotics have demonstrated significant benefit of such treatment to cystic fibrosis (CF) patients suffering from chronic pulmonary infections.

In the US, the most widely used and successful agent in this regard has been tobramycin, which has been shown to produce substantial improvements in lung function and other clinical parameters.

It has now been discovered that inhalable aztreonam lysinate provides successful treatment in cystic fibrosis, bronchiectasis or other suppurative pulmonary disease for pulmonary infections caused by gram-negative bacteria and particularly those caused by antibiotic resistant *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans* and multidrug resistant *Pseudomonas aeruginosa.*

Treatment of these multi-resistant bacterial infections with aerosolized aztreonam lysinate has been successful in eradication of the bacteria as described in Example 2.

Such treatment is either stand alone or may be complementary treatment to other antibiotics, such as tobramycin, which upon extended use, results in the development of anti-tobramycin resistance. When the treatment with tobramycin is interspaced with periods of treatment with aztreonam lysinate, such resistance either does not develop or recedes.

C. Limitations of Current Aerosolized Antibiotics in Treatment of Cystic Fibrosis

To date, an aminoglycoside tobramycin is the only antibiotic with FDA approval for administration as an aerosol. However, despite the benefits obtained in cystic fibrosis patients with administration of aerosolized tobramycin, its utility is somewhat limited.

First, frequent use of aminoglycosides to control pulmonary exacerbations leads to selective development of resistant *Pseudomonas aeruginosa* strains. The widespread emergence of such organisms is acknowledged as a growing crisis in the CF community. For example, 21% of patients screened from 69 different CF centers for the phase III tobramycin clinical trials had isolates resistant to tobramycin (MIC >16 μg/mL). Accordingly, many clinicians are reluctant to prescribe this aerosolized aminoglycoside as chronic suppressive therapy, fearing that it could further promote resistance and thus diminish the effectiveness of IV therapy. In order to reduce the risk of such treatment-emergent resistance, tobramycin therapy is restricted to cycles of 28 days on and 28 days off the drug.

A second limitation of aerosolized tobramycin is its lack of activity against several intrinsically tobramycin resistant bacteria, including *Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and *Burkholderia cepacia,* the latter of which is widely recognized as a significant threat to cystic fibrosis patients. Cystic fibrosis patients infected with *Burkholderia cepacia* have an increased rate of mortality, and many experience a rapid fatal course, as described in *Am. J. Respir. Crit. Care Med.,* 160:1572–1577, (1999). Additionally, *Burkholderia cepacia* is a transmittable infection which can cause epidemic spread among cystic fibrosis patients. Therefore, a patient infected with *Burkholderia cepacia* must be isolated from other patients.

Aerosolized aztreonam lysinate does not induce resistance to aminoglycosides and has good activity against resistant pathogens observed in cystic fibrosis patients.

An aerosolized aztreonam lysinate can either replace tobramycin, or be used as an alternative and intermittent treatment for tobramycin during the 28-day tobramycin free periods, which are required to prevent development of permanent resistence to tobramycin.

Aztreonam lysinate is an antibiotic with excellent activity against many aerobic gram-negative bacteria, including multi-resistant *Pseudomonas aeruginosa.* The spectrum of activity of aztreonam lysinate is similar to that of the aminoglycoside antibiotics tobramycin and gentamycin, and its antipseudomonal activity is comparable to ceftazidine and in several aspects, it is better than tobramycin. For example, aztreonam lysinate is not inhibited by CF patient sputum, making it much more potent drug than tobramycin which is so inhibited.

Aztreonam lysinate resists destruction by most bacterial β-lactamases, which are the source of much treatment-emergent resistance to β-lactam antibiotics frequently appearing among hospitalized patients.

Aztreonam lysinate's activity against gram-negative bacteria, especially *Pseudomonas aeruginosa,* combined with its excellent safety profile makes it a good alternative to aminoglycosides in the treatment of chronic pulmonary infections among cystic fibrosis patients. Thus far, clinical use of aztreonam lysinate in CF patients has included IV administration of aztreonam as single agent therapy or in combination with other antibiotics for treatment of pulmonary exacerbations.

D. Advantages of Aztreonam Lysinate as an Aerosolized Antibiotic

Aztreonam lysinate possesses several features that make it very attractive for aerosol administration to CF patients.

The first of these features stems from its mechanism of action, which, unlike aminoglycoside antibiotics, involves preferential binding to penicillin binding protein 3 (PBP3) and subsequent interference with bacterial cell wall synthesis. Because aztreonam lysinate's mechanism of action differs from that of tobramycin, its use does not contribute to emergence of aminoglycoside-resistant strains of *Pseudomonas aeruginosa.*

The second advantage of an aerosolized formulation of aztreonam lysinate is its activity against tobramycin resistant, and multidrug resistant *Pseudomonas aeruginosa.* When isolates from patients enrolled in the Phase II tobramycin trials were examined, nearly 75% of isolates with a tobramycin MIC >16 μg/mL were susceptible to aztreonam lysinate.

The third feature is aerosolized aztreonam lysinate ability to control intrinsically tobramycin resistant organisms, especially *Burkholderia cepacia,* which is considered resistant to the levels of aztreonam lysinate achieved by parenteral administration.

VII. Antibacterial Activity of Aztreonam

In order to test antibacterial activity of aerosolized aztreonam against multi-resistant strains of *Pseudomonas aeruginosa, Burkholderia cepacia, Stenotrophomonas maltophilia* and *Alcaligenes xylosoxidans,* the in vitro activities of aztreonam in concentrations corresponding to those achievable with inhalable aztreonam were tested against clinical isolates from cystic fibrosis patients.

The aztreonam aerosol delivery according to the invention achieves concentrations of aztreonam to reach levels from 500 to as high as 8000 μg/ml, with an average level around 2,000 μg/ml, of aztreonam in the sputum. These levels depend on the formulation as well as on the nebulizer used for aerosolization. With certain nebulizers the concentration of aztreonam can reach an average level of 5,000 μg/ml.

In vitro determined susceptibilities of the tested bacteria is predictive of clinical efficacy of inhaled aztreonam aerosol or dry powder.

Aztreonam kills by lysing cell walls as long as the local concentration of antibiotic exceeds the bacteria minimal inhibitory concentration (*Med. Clinics N. Am.,* 79: 4, 733–743, (1995)).

The in vitro activity of high aztreonam concentrations against clinical isolates of *B. cepacia, S. maltophilia* and *A. xylosoxidans* was tested at the Children's Hospital and Regional Medical Center in Seattle, Wash. Testing was performed on broth microdilution trays made with 2 fold concentrations of aztreonam from 2 to 2048 μg/mL. *Staphylococcus aureus,* a gram positive organism, was used as a negative control.

Detailed procedure used for testing is described in Example 1. Results are seen in Table 1.

TABLE 1

Organism (# of isolates)	MIC Range	MIC50	MIC90
P. aeruginosa (54)	2–1024	16	512
B. cepacia (38)	2–2048	32	512
S. mallophilia (20)	8–>2048	256	>2048
A. xylosoxidans (20)	2 > 2048	256	2048
S. aureus (20)	512–2048	1024	2048

For testing, each microwell plate contained a 2-fold dilution, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 and 2048 of aztreonam. Each plate containing the microwells was used to test one isolate of one organism.

Table shows the different species of bacteria tested for sensitivity, that is the ability of the antibiotic to inhibit its growth, to aztreonam, with the number of isolates for each species given in parenthesis. The column designated "MIC range" shows the range of the lower and upper limits of sensitivities seen in the tested isolates. The column designated MIC50 shows the median level of sensitivity for the most sensitive 50% isolates. The final column, designated MIC90, shows the median value for the level of sensitivity for the most sensitive 90% of the isolates.

Table 1 shows results of comparative in vitro activity of aztreonam against clinical isolates obtained from cystic fibrosis patients.

For interpretation of this data, the values which represent what concentration of aztreonam is required to inhibit growth of bacteria are compared with the concentrations of aztreonam obtainable by the different routes of administration. Thus, for intravenous administration of aztreonam, the serum level following administration of 2 g of aztreonam, the maximum allowed intravenous dose, the serum level peak is 256 μg/ml and then declines rapidly. At six hours following the administration, the aztreonam level in the serum is in the range of 16 μg/ml. For safety reasons, intravenous aztreonam arginine can only be administered every six hours. With the possible exception of *Pseudomonas aeruginosa* that has a MIC50 of 16 μg/ml, all other organisms would be predominantly resistant to intravenous aztreonam, as their level of resistance exceeds even the peak concentration (256 μg/ml) of serum concentration of sputum of aztreonam following intravenous administration. Since, however, the bacteria resistance is relative to drug concentration, for aerosol administration, the peak concentration should be at least in the 500 to 2000 μg/ml range. Such range is achieved with the doses of aztreonam and the formulation of the invention combined with the efficient nebulizer, according to this invention. At the 500–2000 μg/ml concentration in the sputum, the aerosol therapy according to this invention is able to treat most endobronchial infections caused by gram-negative bacteria, specifically those bacteria listed in Table 1, with exception of *Staphyloccocus aureus.*

The MIC50 and MIC90 have shown that treatment of *P. aeruginosa* with inhalable aztreonam eradicates most *P. aeruginosa* isolates with the high concentrations of aztreonam in sputum of cystic fibrosis patients obtainable after aerosol delivery. The data obtained for *Burkholderia cepacia* isolate indicated that at least half of patients would be expected to respond to such treatment with eradication of the bacteria. If sufficiently high concentrations of aztreonam are delivered to the lung, the percentage is expected to be higher. Since the *Burkholderia cepacia* infection is now viewed as a largely untreatable condition, treatment with inhalable aztreonam by aerosol is the first documented efficacious therapy.

The results obtained in these studies are surprising and unexpected as there is no indication in the literature that *Burkholderia cepacia* is susceptible to treatment with aztreonam. The data also shows that some isolates of *S. maltophilia* and *A. xyloxidans* respond to high concentration of aztreonam.

Inhalation of aztreonam according to the invention permits reaching concentrations of aztreonam in the sputum as high as 2000–5,000 μ/mL. The sputum aztreonam levels achieved via aerosol administration exceed those required to inhibit organisms responsible for otherwise untreatable infections in CF patients.

Furthermore, aztreonam delivered by inhalation to all patients with *Burkholderia cepacia* and/or *S. maltophilia* and/or *A. xyloxidans* together with other antibiotics whether administered systemically parenterally or by inhalation contributes to synergy of such treatment. A combination of inhalable aztreonam with other antibiotics provides another therapeutic approach to treat multi-resistant bacterial strains.

The studies described herein demonstrated that the concentrations of aztreonam achieved following aerosol administration have activity against *Burkholderia cepacia* isolated from CF patients' sputum as well as against other bacteria which are largely resistant to treatment with other antibiotics.

The MIC50 and MIC90 observed for a gram positive bacteria, *Staphylococcus aureus,* show that high concentrations of aztreonam had some activity against this gram positive bacteria. These findings, however, have no great significance as there are many other drugs with reasonable efficacy against *Staphyloccocus aureus.*

VIII. Safety and Clinical Testing

The infections requiring particular attention are infections caused by and include *B. cepacia, S. maltophilia* and *A.*

xylosoxidans, as well as multi-resistant strains of *Pseudomonas aeruginosa*. The most clinical significant infection is the former.

In order to determine if an appropriately formulated aztreonam lysinate for aerosolization could become effective for treatment of these rare but very resistant bacterial strains, the treatment with aerosolized aztreonam lysinate was initiated and tested in a cystic fibrosis patient having a severe *Burkholderia cepacia* infection which did not respond to any treatment. The clinical treatment and results obtained with an aerosolized aztreonam lysinate is described in Example 2.

Safety of the aztreonam lysinate formulation was also studied both in man and in Beagle dog. Conditions of these studies are described in Samples 11 and 12.

Results of both studies confirm the safety of the aztreonam lysinate formulation for inhalation. As compared to a formulation containing arginine, the new formulation is safe in man (Example 10) and in dog at up to 200 fold of the human dose shown in a 28 day dog study (Example 11). Increased safety establishes utility of the aztreonam lysinate in both instances.

Safety results from both studies show that there were no serious adverse events recorded during the trial and no subject was withdrawn from the trial because of an adverse event. In total, 7 post-dose adverse events were reported for 7 subjects. No single adverse event was experienced by more than one subject. A single drug-related adverse event occurred in each of the 95 and 190 mg inhaled aztreonam dose groups (headache and dizziness, respectively) and 2 drug-related adverse events occurred in the 285 mg inhaled aztreonam dose group (dysgeusia, i.e. unpleasant taste and cough). One adverse event was of Grade 2 severity (headache) and the remaining adverse events were of Grade 1 severity. All adverse events resolved before the end of the trial. The adverse event of cough led to discontinuation of the trial medication, although the subject continued in the trial and completed all trial assessments.

There were no notable mean changes from baseline in any post-dose pulmonary function parameter. One subject, who was dosed with placebo, had an FEV_1 decrease from baseline of greater than 15% (+30 min). This was recorded as an adverse event, but was not considered to be related to the trial medication.

There were no notable mean changes from baseline in any hematology or coagulation parameter assessed.

There were no notable mean changes from baseline in systolic and diastolic pressure, pulse rate, oral temperature, respiration rate or pulse oximetry in subjects dosed with placebo or 90 mg, 190 mg or 285 mg inhaled aztreonam. No individual subject value in any of these parameters was reported as an adverse event.

There were no notable mean changes from baseline in any ECG parameter assessed and no individual subject ECG value was reported as an adverse event. No changes from baseline were noted on any post dose physical examination.

In conclusion, inhaled aztreonam was generally safe and well tolerated when administered at does of 95 mg, 190 mg and 285 mg in this trial.

There were no clinically significant changes in FEV_1 (defined as a decrease from baseline of 15% or more) in any subject treated with aztreonam. One subject who was treated with placebo experienced a decrease from baseline in FEV_1 of 15.58%. This was reported as an adverse event not considered to be related to treatment. There were no clinically significant changes in any other safety measurement (in either mean or individual values) there were considered to be treatment-related.

The objective of the second study was to assess the tolerability and toxicity of aerosolized aztreonam lysinate formulation in the Beagle dog after 28 day repeat dosing by the inhalation route and to evaluate the reversibility of any effects after a 14 day recovery period. Inhalation exposure was undertaken using a closed face-mask system with the dogs breathing passively from an ultrasonic nebulizer.

Conditions under which the study was conducted are described in Example 11.

Overall results of this study show that the inhalation of nebulized aztreonam lysinate is safe and there were no observed adverse clinical signs or treatment related effects on body weight, food consumption, ophthalmoscopic findings, ECG readings, laboratory investigations or organ weights.

There were no necropsy or histological findings that could be attributed to treatment with Aztreonam. Since the anticipated human dose is 75 mg, and the average weight is 75 kg, the safety margin may be as high as 200 fold over the human dose.

UTILITY

The method of treatment and the inhalable aztreonam lysinate compositions disclosed herein is suitable for treatment of respiratory tract infections caused by *Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans,* and multidrug resistant *Pseudomonas aeruginosa* as well as for treatment of other pulmonary infections caused by gram-negative bacteria.

EXAMPLE 1

In vitro Testing of Isolates from Cystic Fibrosis Patients

This example describes procedure used for in vitro studies of bacterial isolates obtained from cystic fibrosis patients.

Bacterial respiratory tract isolates (144) from patients with CF that had been stored at −70° C. were cultivated by two consecutive overnight passages at 37° C. on 5% blood agar (Remel, Lenexa, Kans.).

Minimal inhibitory concentrations (MIC's) were determined by the following steps:

MIC Antimicrobial Testing Aerobic Organisms

1. MIC trays were brought to room temperature.

2. 3.0 ml physiological saline was inoculated with an 18–24 h culture of organism to be tested to a turbidity equal to a 0.5 McFarland Standard (1.5×10^8 CFU/ml). This corresponds to an OD600 of 80–88% transmission.

3. Within 15 minutes of preparation, the adjusted inoculum suspension was diluted by transferring 100 ml into a 2.9 ml diluent of sterile water.

4. The suspension was gently mixed by inversion and 10 ml was dispensed into each MIC well having initial volume of 100 µl. The final concentration in each well was equal to 5×10^5 CFU/ml or 5×10^4 CFU/well.

5. Trays were incubated aerobically at 37° C. for 16–20 hours. The same incubation temperature was maintained for all cultures. Microdilution trays were not stacked more than four high.

6. Antimicrobial endpoint was read and recorded as the first well showing no readily visible growth or haze as detected by the unaided eye.

7. The microdilution trays were contacted with 2 fold concentrations of aztreonam lysinate from 2 to 2048 mg/mL. Each microwell plate was treated with a 2-fold dilution of aztreonam lysinate in following amounts: 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 and 2048 μg/ml. Each plate containing the microwells was used to test one isolate of one organism.

8. Results were read and recorded.

EXAMPLE 2

Clinical Treatment of Patient with *Burkholderia cepacia*

This example describes a first finding of efficacy of the aerosolized aztreonam treatment of a cystic fibrosis patient suffering from resistant *Burkholderia cepacia.*

The patient was a 20-year-old female with cystic fibrosis and end stage lung disease. She had been diagnosed with *Burkholderia cepacia* pulmonary infections that had become resistant to all known intravenous, oral and inhaled antibiotics. She had two-documented genetically different strains of *Burkholderia cepacia.* For this reason the patient was rejected as a candidate for a lung transplant.

The patient was provided with a formulation of the invention comprising 200 mg/ml of aztreonam and instructed to use this formulation in 3 to 5 ml of diluent and use it in an air compressor powered breath enhanced jet nebulizer and take the therapy twice a day. This type of nebulizer only delivers about 10 to 20% of the dose placed in the nebulizers to the lungs, however, that was only nebulizer available to the patient for home treatment.

After three months of continuous twice a day therapy, the pulmonary infection was successfully treated and no evidence of *Burkholderia cepacia* could be detected. The patient was considered treated from the infection and eventually underwent a successful lung transplant procedure.

There was no postoperative reoccurrence or relapse of the *Burkholderia cepacia* infection despite of intensive immunosuppression therapy following the transplantation.

These findings were surprising since previous use of commercially available aztreonam arginine in an older generation delivered in even less efficient nebulizers did not lead to eradication of *P. aeruginosa* as described in *Clinics Chest Med.,* 19:473–86, (September 1998). In the trial described there, the authors stopped therapy at the development of any aztreonam resistance rather than continuing treating these patients. Prior work did not test or speculate that this therapy could be effective in treating other gram negative bacteria including *Burkholderia cepacia, S. maltophilia, X. xylosoxidans,* or other multidrug resistant pseudomonas infections.

The results obtained with treatment of the above patient are even more surprising in that the eradication of *Burkholderia cepacia* is extremely rare occurrence, particularly when the infection is well established as was in the case of this patient.

EXAMPLE 3

Preparation of Aztreonam Lysinate Dry Powder

This example provide methods and procedures used for preparation of aztreonam lysinate containing inhalable dry powder.

For dry powder formulation of the invention, a purified aztreonam lysinate is milled to a powder having mass median average diameters ranging from 1 to 5μ by media milling, jet milling, spray drying, or particle precipitation techniques.

Particle size determinations is made using a multi-stage Anderson cascade impactor.

Media milling may be accomplished by placing the drug into a mill containing, for example, stainless steel or ceramic balls and rotating or tumbling the material until the desired drug particle size ranges are achieved.

Jet milling uses very high pressure air streams to collide particles with one another, with fine particles of the desired size being recovered from the mill.

Spray drying is achieved by spraying a fine mist of drug solution onto a support and drying the particles. The particles are then collected.

Particle precipitation is achieved by adding a co-solvent to spray dried particles. The solubility of the drug falls to the point where solid drug particles are formed. The particles are collected by filtration or centrifugation. Precipitation has the advantage of being highly reproducible and can be performed under low temperature conditions, which reduce degradation.

EXAMPLE 4

Dry Powder Inhalers

Metered dose and the dry powder formulations of the invention may be used directly in metered dose or dry powder inhalers.

A metered dose inhaler consists of three components: a canister containing the propellant drug suspension, a metering valve designed to deliver accurately metered volumes of the propellant suspension, and an oral adapter which contains a spray orifice from which the metered dose is delivered. In the rest position, the metering chamber of the valve is connected to the drug suspension reservoir via a filling groove or orifice. On depression of the valve this filling groove is sealed and the metering chamber is exposed to atmospheric pressure via the spray orifice in the oral adapter and the valve stem orifice. This rapid pressure reduction leads to flash boiling of the propellant and expulsion of the rapidly expanding mixture from the metering chamber. The liquid/vapor mixture then enters the expansion chamber which is constituted by the internal volume of the valve stem and the oral adapter. The mixture undergoes further expansion before being expelled, under its own pressure, from the spray nozzle. On exit from the spray orifice, the liquid ligaments which are embedded in propellant vapor are torn apart by aerodynamic forces. Typically, at this stage, the droplets are 20 to 30μ in diameter and are moving at the velocity of sound of the two-phase vapor liquid mixture (approximately 30 meters per second). As the cloud of droplets moves away from the spray nozzle, it entrains air from its surroundings and decelerates, while the propellant evaporates through evaporation and the entrained droplets eventually reach their residual diameter.

At this point, the particles/droplets consist of a powdered drug core coated with surfactant. Depending on the concentration and the size of the suspended material the powdered drug core consists of either individual drug particles or aggregates. Currently, meter dose inhaler technology is optimized to deliver masses of 80 to 100 micrograms of drug, with an upper limitation of 1 mg of drug deliverable.

An alternated route of dry powder delivery is by dry powder inhalers. There are two major designs of dry powder inhalers, device-metering designs in which a reservoir of

drug is stored within the device and the patient "loads" a dose of the device into the inhalation chamber, and factory-metered devices in which each individual dose has been manufactured in a separate container. Both systems depend upon the formulation of drug into small particles of mass median diameters from 1 to 5 microns, and usually involve co-formulation with large excipient particles (typically 100 micron diameter lactose particles). Drug powder is supplied into the inhalation chamber (either by device metering or by breakage of a factory-metering dosage) and the inspiratory flow of the patient accelerates the powder out of the device and into the oral cavity. Non-laminar flow characteristics of the powder path cause the excipient-drug aggregate to decompose, and the mass of the large excipient particles causes their impaction at the back of the throat, while the inhaler drug particles are deposited deep in the lungs. Current technology for dry powder inhalers is such that payload limits are around 50 mg of powder (of which drug is usually a partial component by mass). Excipients commonly used are lactose, however in the current case aztreonam is reacted with amino acid lysine and such reaction leads to a better powder formation and more stable powder formulation.

Effective dosage levels of aztreonam lysinate antibiotic for dry powder inhalation and metered dose inhalation result in the delivery of at least about 25 mg, and more preferable about 50 to about 100 mg of aztreonam lysinate to the lung of the patient receiving treatment. Depending on the efficiency of the dry powder delivery device, dry powder formulations suitable for use in the invention comprise from about 1.0 to about 250 mg, preferably from about 10 to about 100 mg of powder in an amorphous or crystalline state in particle sizes between 1 and 5 microns in mass median average diameter necessary for efficacious delivery of the antibiotic into the endobronchial space.

EXAMPLE 5

Preparation of Aztreonam Lysinate Salt

This example describes procedure used for preparation of aztreonam lysinate salt.

To a solution of 10 g (23 mmol) of aztreonam lysinate in 100 mL of MeOH cooled in an ice bath was added dropwise 23 mL (23 mmol, 1.0 eq) of 1N sodium hydroxide solution. The resulting solution was warmed to ambient temperature over a period of 30 min, and then the solvent was removed under reduced pressure. Diethylether (50 mL) was added and the slurry concentrated. This step was repeated four times to provide a yield of 10.1 g (96%) of aztreonam lysinate salt as a white powder.

EXAMPLE 6

Formulation and Spray Drying of Aztreonam (from Alpha Form) Lysinate

Aztreonam (alpha form, 29.4 g with 15% moisture, equivalent to 25.0 g anhydrous) was suspended and rapidly stirred in water (190 mL) and cooled with a crushed ice bath. L-Lysine (anhydrous, 17.7 g, dissolved in 40 mL of room temperature water) was titrated over 6 minutes to the milky white suspension to obtain a pH of 4.34. The total volume of the aztreonam lysinate solution was approximately 270 mL and had a yellow to light brown color. Approximately 1 g of charcoal was added to the stirring solution and was then filtered. The aztreonam lysinate solution was kept at 2 to 10° C. Spray drying was accomplished giving a yield of 22.2 g (56%) of aztreonam lysinate. Below illustrates an unoptimized method for spray drying:

Inlet Set 135° C.

Aspirator 90% (a value of 100%=35 cubic meters/hr).

Pump 34% (a value of 100%=1500 mL/hr).

Ar flow at nozzle 400 L/hr initial; at middle of run increased to 600 L/hr.

Receiver flask temp 35 to 40° C.

EXAMPLE 7

Testing Nebulizers

This example describes testing of nebulizers in clinical conditions to determine dose to be used in each.

A clinical study is conducted in order to determine the concentration of aztreonam lysinate in the aerosol formulation required to achieve a sputum concentration between 500 μg/gm and 2000 μg/gm sputum at 10 min post-completion of aerosol administration using an atomizing, ultrasonic or jet nebulizer.

In this study, cystic fibrosis patients receive serial escalating doses of multiple of 75 mg aztreonam lysinate (1 ml of a 75 mg/ml solution in ¼ NS) from each of the nebulizers. The doses are separated by at least 2 days and not more than 5 days. Peak serum and sputum concentrations are assessed.

EXAMPLE 8

Testing of Sputum Inhibitory Activity

This example describes conditions used for testing inhibitory activity of aztreonam lysinate and tobramycin on sputum or hog gastric mucin.

Reagents

Unless stated otherwise, all chemicals were purchased from Sigma Chemical Company (St. Louis, Mo.), and all solutions were prepared in sterile deionized water. Aztreonam (Azactam®) were obtained from Elan Biopharmaceuticals. Aztreonam lysinate was prepared at Corus Pharma, Seattle, Wash. Working stock solutions of aztreonam and aztreonam lysinate were prepared in sterile deionized water and used immediately.

Culture Medium

Divalent cation adjusted Mueller Hinton broth (CAMHB) was purchased from PML and used both as the, study growth medium for *P. aeruginosa* and as the assay growth medium.

Sputum

Sputum was obtained from children and adults with CF who were not receiving any other antimicrobial drug for at least 48 hours prior to the collection of the sample. Sputum was sterilized by stirring with a magnetic stirrer under UV light for 4 hours. Sterility was tested by inoculating 100 uL of sputum into 10 mLs of CAMHB a row medium and incubating overnight. Resulting culture was examined for turbidity and 100 muL were plated on Luria agar to ensure sterility. The sputum samples were kept frozen at −20° C. until used.

Organisms

Fresh subcultures of *P. aeruginosa* strain PA27853 were used for each experiment. Freezer stock was grown on Luria agar plates (Sigma L-3522) overnight at 37° C. A single colony was picked and inoculated into 5 mL of CAMHB and grown for 16 hours at 37° C. with shaking at 250 rpm. This overnight culture was diluted 1:10,000 in fresh CAMHB or

in fresh CAMHB supplemented with 10% (w/v) porcine gastric mucin (Sigma M-1778), then autoclaved, or 1% sterilized CF sputum.

Killing Curves

P. aeruginosa (initial density ~10^6 CFU/mL) was grown in overnight culture and diluted 1:10,000 in broth. The dilutions were each divided into 4 tubes (10 mL per tube) and antibiotic was added to each tube to a final concentration of 0, 0.1, 1, and 10 times the MIC for strain PA27853 (4 μg/mL for aztreonam, 1.56 μg/mL for tobramycin, determined by standard methods). Each tube was incubated at 37° C. with 250 rpm shaking. Each hour, samples were removed from the tube, diluted, and plated on Luria agar for quantitation. Plates were incubated overnight at 37° C. and colonies were counted by hand.

EXAMPLE 9

Clinical Trial Protocol

This example describes a protocol used for clinical trial and to compare the pharmacokinetics of increasing dosage of an aztreonam lysinate formulation administered by the PARI electronic nebulizer to patients with cystic fibrosis.

The primary aim of this study was to determine which of the tested dose levels delivered by aerosol can deliver sufficient amount of aztreonam lysinate to achieve a mean peak sputum aztreonam lysinate concentration of 1000 μg/gm or greater measured 10 minutes after the completion of nebulization in patients with CF.

The secondary aim was to determine whether the aztreonam lysinate concentration required to achieve a mean peak sputum concentration of 1000 μg/gm or greater is safe and well tolerated by the patient.

Study Design

This was an open label, multicenter, randomized, dose escalation study.

Each arm contained different dose. Two arms delivered the same aztreonam lysinate formulation.

1. 1.0 ml of aztreonam lysinate solution of 75 mg/ml
2. 2.0 ml of aztreonam lysinate solution of 75 mg/ml
3. 3.0 ml of aztreonam lysinate solution of 75 mg/ml

Efficacy and Safety Assessment

In this study, the following efficacy and safety parameters that were assessed were:

The efficacy was determined for each nebulizer by measuring concentration of aztreonam lysinate in sputum 10 minutes after completion of nebulization. Mean concentration of 1000 μg/gm of sputum was considered adequate.

The safety parameters assessed:

1. Incidence of treatment related adverse reactions occurring during the administration of the aerosolized aztreonam lysinate at the different dose levels.
2. Acute bronchospasm at the time of drug administration.
3. Absorption of aztreonam lysinate into the systemic circulation.

Each patient received in random order at least one administration. Each aerosol administration was separated by a minimum of 48 hr. Sputum samples were collected at baseline, 1, 2, 4 and 6 hours post-completion of the aerosol drug administration to measure aztreonam lysinate concentration. Serum samples were collected at baseline, 1, 2, 4 and 6 hours post-completion of aerosol administration to measure aztreonam lysinate levels.

Airway irritation and acute bronchospasm were assessed by measuring spirometry immediately prior to and 30 min post-completion of aerosol administration. A decrease in forced expired volume in one second (FEV1) >15% in the 30 min spirometry test is considered evidence of bronchospasm.

Additional objectives of this study were to determine and at what dose the PARI electronic nebulizer tested can aerosolize sufficient aztreonam lysinate sulfate to achieve a mean peak sputum aztreonam lysinate concentration of 1000 μg/gm or greater in at least 85% of patients with CF measured 10 minutes after the completion of nebulization to determine whether the aztreonam lysinate concentration required to achieve a mean peak sputum concentration of 1000 μg/gm or greater is safe and well tolerated by the patient. Safety is defined as a lack of acute bronchospasm and minimal systemic absorption.

Patient Treatment

All patients with underlying disease of cystic fibrosis (CF), confirmed at entry by the inclusion/exclusion criteria specified in this protocol, were eligible for enrollment into the study. Investigators at the participating CF centers selected patients that meet all of the inclusion criteria and one of the exclusion criteria.

Eligible patients were admitted to the study center on the day of the study and receive aerosol therapy if they fulfilled entrance criteria.

Physical exam is administered by a physician or RC nurse prior to initial aerosol treatment only.

Vital signs, height, weight, oximetry, assessment of current respiratory status and brief medical history were used. Sputum and serum samples were collected to measure baseline aztreonam lysinate concentrations.

Patients were sitting upright and use nose clips during the aerosol administration.

The total duration of time and the number of inhalations required to complete the aerosol treatment were recorded.

Any evidence of wheezing or respiratory distress are recorded as well as number of rest periods required by the subject because of dyspnea or excessive coughing during the administration period.

Immediately after completing the aerosol therapy, the subject rinsed with 30 ml of normal saline through the mount, gargled for 5–10 seconds and expectorated the rinse. This was repeated for a total of three rinses.

Sputum specimens were collected at 10 minutes after rinsing oral cavity and 2 hours after completion of the aerosol drug administration.

Serum was collected at 1 and 2 hours after completion of the aerosol drug administration for determination of the aztreonam lysinate levels.

Spirometry was obtained 30 minutes following completion of the aerosol drug administration.

Following the last aerosol treatment of the study, patients received a brief physical exam after post-spirometry has been measured.

EXAMPLE 10

Safety Clinical Trials

This example describes clinical protocol used for safety clinical trial with aztreonam lysinate.

Name of Finished Product: Aztreonam for Inhalation

Name of Active Ingredient: Aztreonam lysinate.

This was a randomized, double-blind, placebo controlled trial to assess the safety and tolerability of inhaled aztreonam lysinate in healthy male and female volunteers.

The primary objective was to determine the safety and tolerability of 3 escalating doses of aztreonam for inhalation in male and female volunteers.

Methodology

Subjects were screened for inclusion in the trial up to 21 days before dosing and their eligibility was confirmed at the day 1 visit. Subjects were admitted to the clinic in the morning on the day before dosing (Day −1). Within each of the 3 treatments groups receiving 95 mg, 190 mg and 285 mg inhaled aztreonam, subjects were allocated randomly to either active treatment (6 subjects) or to placebo (2 subjects). Progression to the 190 mg and 285 mg doses occurred only when blinded safety data from the 95 mg and 190 mg groups, respectively, had been assessed. On the morning of day 1, subjects self-administered their allocated trial medication by inhalation using an eFlow™IMP nebulizer (PARI). Subjects remained in the clinic for 24 h after dosing and returned 3 days after dosing for a follow-up visit. Safety was monitored throughout the trial.

Number of Subjects

24 subjects (3 groups of 8 subjects) were recruited and 24 were included in the safety analysis.

Diagnosis and Main Criteria for Inclusion

Subjects were male or female non-smokers, aged 18 to 55, weighing between 50 and 100 kg with a body mass index of 18 to 28 $kg.m^{-2}$, with a negative Coombs' test result and a forced expiratory volume in one second (FEV_1) of at least 80% of the predicted normal.

Test Product, Dose and Mode of Administration

Placebo (1, 2 or 3 ml sterile 0.9% saline; manufactured by Phoenix Pharma, was self-administered by the subject into the airways using an eFlow™IMP nebulizer (PARI).

Safety

Adverse events, laboratory data (hematology, clinical chemistry, Coombs' test, coagulation and pregnancy test for women of childbearing potential), urinalysis, vital signs, ECG, physical examination (including chest auscultation) and pulmonary function tests.

Safety Results

No serious adverse events were recorded during the trial and no subject was withdrawn from the trial because of an adverse event.

EXAMPLE 11

Beagle Dog Safety Study

This example describes conditions used for Beagle dog safety studies.

Sixteen male and female Beagle dogs were allocated to 4 dose groups and treated as follows:

Dose Group/	Target Dose Levels ($mg . kg^{-1} . day^{-1}$)		Animal Numbers/Allocation		
Treatment	Total	Pulmonary		Males	Females
1-Vehicle Control	0	0	Main Study	1–3	17–19
			Recovery	4–5	20–21
2-Low Dose	40	8	Main Study	6–8	22–24
3-Intermediate Dose	80	16	Main Study	9–11	25–27
4-High Dose	200	40	Main Study	12–14	28–30
			Recovery	15–16	31–32

During the pretrial and recovery phases of the study animals were monitored at least once daily for any adverse clinical signs. During the treatment period, all animals were examined for any adverse clinical signs before exposure, continuously during exposure and at cca 1–2 h after exposure. Body weights were recorded weekly whilst food consumption was monitored daily up until the end of the study period.

Ophthalmoscopic examinations were undertaken once pretrial, during Week 4 of treatment and towards the end of the 14 day recovery period for designated animals. Electrocardiograms were recorded once pretrial, on Days 2 and 28 of treatment and from designated recovery animals towards the end of the 14 day recovery period.

Blood and urine samples for routine hematology, clinical chemistry and urinalysis investigations were obtained from all animals once pretrial, during Week 4 of treatment, and from designated recovery animals towards the end of the 14 day recovery period. Blood samples for toxicokinetic analysis were collected from all animals from Groups 2, 3 and 4 on Days 1 and 27 of exposure at the following target timepoints: predose, immediately post dose (IPD) and at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post dose. Samples were collected from Group 1 animals predose and immediately post dose. Urine samples for toxicokinetic analysis were collected from all animals on Days 1 and 27 of exposure over a 24 h period.

On completion of the 28/29 day treatment period or 14 day recovery period, all animals were subjected to a detailed necropsy with recording of organ weights. Microscopic evaluation was undertaken on a comprehensive list of tissues.

Overall estimated mean achieved doses of 0, 53.0, 94.3 and 194.7 $mg.kg^{-1}.day^{-1}$ (estimated mean pulmonary doses of 0, 10.6, 18.9 and 38.9 $mg.kg^{-1}.day^{-1}$) were achieved for Groups 1, 2, 3 and 4, respectively. Particle size distribution measurements indicated the Aztreonam aerosol was respirable for dogs.

Treatment described herein was safe method for any sign of adverse reaction.

What is claimed is:

1. An inhalable composition comprising aztreonam lysinate, said composition suitable for the treatment of pulmonary bacterial infections caused by gram-negative bacteria, wherein said aztreonam lysinate is prepared as an inhalable dry powder having a particle size with a mass medium average diameter from about 1 to about 5μ.

2. The composition of claim **1** wherein the aztreonam lysinate is alpha aztreonam lysinate.

3. The composition of claim **1** wherein the gram-negative bacteria is *Burkholderia cepacia.*

4. The composition of claim **1** wherein the gram-negative bacteria is *Stenotrophomonas maltophilia.*

5. The composition of claim **1** wherein the gram-negative bacteria is *Alcaligenes xylosoxidans.*

6. The composition of claim **1** wherein the gram-negative bacteria is a multidrug resistant *Pseudomonas aeruginosa.*

7. The composition of claim **1** comprising from about 1 to 250 mg of the aztreonam lysinate, wherein the composition may be administered as the inhalable dry powder by a dry powder inhaler or as a diluted saline solution by a metered dose inhaler the aerosolable solution.

8. The composition of claim **7**, comprising 10 to 100 of aztreonam lysinate.

9. The composition of claim **8** comprising 75 mg of aztreonam lysinate, wherein said composition may be administered twice or three times a day.

10. The composition of claim 7 wherein the aztreonam lysinate is alpha aztreonam lysinate prepared from an alpha aztreonam form.

11. The composition of claim 10 wherein said alpha aztreonam lysinate has impurity lower than 1% and stability for at least two years.

12. The composition of claim 11 wherein said alpha aztreonam lysinate contains less than 100 ppm of residual alcohol and initial levels of contaminants generated from the alpha aztreonam lysinate are less than 1%.

13. The composition of claim 10 wherein said aztreonam lysinate is in a solution comprising a volume of saline from about 1 to about 5 ml, said saline comprising between about 0.09% and about 0.9% of chloride, w/v, or an equivalent amount of bromine or iodine, wherein said solution is aerosolable and wherein said aerosolable solution has a pH from about 4.2 to about 7.5.

14. The composition of claim 13 wherein said saline comprises from about 0.1 to about 0.45% of sodium chloride, w/v, and wherein said pH is from about 5.5 to about 7.

15. The composition of claim 14 wherein the aztreonam lysinate is present in a concentration of about 75 mg/ml in said saline.

16. A method for administering aztreonam lysinate comprising administration of the composition of claim 7 by a dry powder inhaler or by a metered dose inhaler, wherein said composition may be administered one to twelve times a day, provided that if the composition is delivered more than twice a day, a total dose of aztreonam lysinate is not higher than 750 mg a day.

* * * * *

EXHIBIT 6

TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING SECOND APPLICATION

Docket No. 3818.02-5

In re Application of: ALAN BRUCE MONTGOMERY

Application No. 10/613,639

Filed: JULY 3, 2003

For: INHALABLE AZTREONAM LYSINATE FORMULATION FOR TREATMENT AND PREVENTION OF PULMONARY BACTERIAL INFECTIONS

The owner, CORUS PHARMA, INC. of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application, which would extend beyond the expiration date of the full statutory term defined in 35 U.S.C. 154 to 156 and 173 as shortened by any terminal disclaimer filed prior to the grant of any patent granted on pending second Application Number 10/654,815, filed on SEPTEMBER 4, 2003. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the second application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term as defined in 35 U.S.C. 154 to 156 and 173 of any patent granted on the second application, as shortened by any terminal disclaimer filed prior to the patent grant, in the event that any such granted patent: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims cancelled by a reexamination certificate, is reissued, or in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

Check either box 1 or 2, if appropriate.

1. ☐ For submissions on behalf of an organization (e.g., corporation, partnership, university, government agency, etc.), the undersigned is empowered to act on behalf of the organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

09/25/2006 YPOLITE1 00000047 10613639
01 FC:2814 65.00 OP

2. ☒ The undersigned is an attorney of record.

3. Owner/applicant is ☒ Small entity ☐ Large entity

The terminal disclaimer fee under 37 CFR 1.20(d) is $65.00 and is to be paid as follows:

☒ A check in the amount of the fee is enclosed.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 16-1331.

☐ Payment by credit card. Form PTO-2038 is attached.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

PTO suggested wording for terminal disclaimer was

☒ unchanged. ☐ changed (if changed, an explanation should be supplied.)

Dated: SEPTEMBER 21, 2006

Signature

Name and Address of Person Signing

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I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to "Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450" [37 CFR 1.8(a)] on SEPTEMBER 21, 2006 (Date)

Signature of Person Mailing Correspondence

MELINDA TOMPKINS

Typed or Printed Name of Person Mailing Correspondence

TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING SECOND APPLICATION

Docket No. 3818.02-5

In re Application of: ALAN BRUCE MONTGOMERY

Application No. 10/613,639

Filed: JULY 3, 2003

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In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term as defined in 35 U.S.C. 154 to 156 and 173 of any patent granted on the second application, as shortened by any terminal disclaimer filed prior to the patent grant, in the event that any such granted patent: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims cancelled by a reexamination certificate, is reissued, or in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

Check either box 1 or 2, if appropriate.

1. ☐ For submissions on behalf of an organization (e.g., corporation, partnership, university, government agency, etc.), the undersigned is empowered to act on behalf of the organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

09/25/2006 YPOLITE1 00000047 10613639
02 FC:2814 65.00 OP

2. ☒ The undersigned is an attorney of record.

3. Owner/applicant is ☒ Small entity ☐ Large entity

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PTO suggested wording for terminal disclaimer was

☒ unchanged. ☐ changed (if changed, an explanation should be supplied.)

Dated: SEPTEMBER 21, 2006

Signature

Name and Address of Person Signing

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I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to "Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450" [37 CFR 1.8(a)] on SEPTEMBER 21, 2006 (Date).

Signature of Person Mailing Correspondence

MELINDA TOMPKINS

Typed or Printed Name of Person Mailing Correspondence

EXHIBIT 7

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,214,364 B2
APPLICATION NO. : 10/613639
DATED : May 8, 2007
INVENTOR(S) : Alan Bruce Montgomery

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At column 5, line 26, delete "lysinate".

At column 6, line 54, delete "lysinate".

At column 13, line 55, insert -- of aztreonam -- between "mg/mL" and ",".

At column 13, line 56, insert -- of aztreonam -- between "mg/mL" and ",".

At column 16, line 42, delete "lysinate".

At column 16, line 49, delete "lysinate".

At column 16, line 60, delete "lysinate".

At column 16, line 61, delete "lysinate".

At column 17, line 2, delete "lysinate".

At column 36, line 24, delete "lysinate".

At column 37, line 40, after the word "solution", delete "of" and insert therefor -- containing --.

At column 37, line 40, insert -- of aztreonam -- after "mg/ml".

At column 37, line 41, after the word "solution", delete "of" and insert therefor -- containing --.

At column 37, line 41, insert -- of aztreonam -- after "mg/ml".

At column 37, line 42, after the word "solution", delete "of" and insert therefor -- containing --.

At column 37, line 42, insert -- of aztreonam -- after "mg/ml".

At column 40, line 47, i.e., the 6th line of Claim 1, "5μ" should read -- 5μm --.

At column 40, line 60, i.e., the third line of Claim 7, "may be" should read -- is --.

At column 40, line 60, i.e., the third line of Claim 7, "the" should read -- an --.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,214,364 B2
APPLICATION NO. : 10/613639
DATED : May 8, 2007
INVENTOR(S) : Alan Bruce Montgomery

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At column 40, line 61, i.e., the 4th line of Claim 7, after the word "as", "a" should read -- an aerosolable --.

At column 40, line 62, i.e., the 5th line of Claim 7, delete "the aerosolable solution".

At column 40, line 63, i.e., the first line of Claim 8, insert -- mg -- between "10 to 100" and "of".

At column 40, line 65, i.e., the first line of Claim 9, after the word "claim", delete "7" and insert therefor -- 8 --.

At column 40, line 66, i.e., the second line of Claim 9, delete "lysinate".

At column 41, line 14, i.e., the 4th line of Claim 13, insert -- sodium -- between "of" and "chloride".

At column 42, line 6, i.e., the second line of Claim 15, delete "lysinate".

Signed and Sealed this

Third Day of June, 2008

JON W. DUDAS
Director of the United States Patent and Trademark Office

EXHIBIT 8

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Patent Number:	7214364	**Application Number:**	10613639
Issue Date:	05/08/2007	**Filing Date:**	07/03/2003
Window Opens:	05/08/2010	**Surcharge Date:**	11/09/2010
Window Closes:	05/09/2011	**Payment Year:**	
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EXHIBIT 9

Cayston IND and NDA Submissions and Correspondence

Date	Doc From	Doc To	Doc Description	Submission and/or Serial Number	Vol.
4/14/2003	Corus	FDA	1571, TOC, Intro Statement, General Investigational Plan, IB, Protocol, PI Info	IND 0000	1 of 9
4/14/2003	Corus	FDA	Clinical references, F	IND 0000	1b of 9
4/14/2003	Corus	FDA	Clinical references, G-N	IND 0000	1c of 9
4/14/2003	Corus	FDA	Clinical references, O-W	IND 0000	1d of 9
4/14/2003	Corus	FDA	CMC	IND 0000	2 of 9
4/14/2003	Corus	FDA	CMC References	IND 0000	2a of 9
4/14/2003	Corus	FDA	NonClinical Pharmacology and Toxicology Information, Attachment 8.1	IND 0000	3 of 9
4/14/2003	Corus	FDA	NonClinical attachments, 8.1.1-8.3.1	IND 0000	4 of 9
4/14/2003	Corus	FDA	NonClinical attachments, 8.4, 8.4.1	IND 0000	5 of 9
4/14/2003	Corus	FDA	NonClinical attachments, 8.4.1.1-8.9	IND 0000	6 of 9
4/14/2003	Corus	FDA	NonClinical references, A-B	IND 0000	6a of 9
4/14/2003	Corus	FDA	NonClinical references, C-S	IND 0000	6b of 9
4/14/2003	Corus	FDA	Previous Human Experience, Attachment 9.1	IND 0000	7 of 9
4/14/2003	Corus	FDA	Previous Human Experience, Attachment 9.1 continued	IND 0000	8 of 9
4/14/2003	Corus	FDA	Previous Human Experience, Attachment 9.1 continued	IND 0000	9 of 9
4/15/2003	Corus	FDA	Response to Division's letter dated 1/17/03, re: additional information on microbiology components of development plan	IND	
4/22/2003	FDA	Corus	Telephone contact log re. FDA assigns IND number 64,402	IND	
4/24/2003	FDA	Corus	Pharm/Tox comments re: carcinogencity study	IND	
4/28/2003	Corus	Corus	Internal Memo re: AI IND submission, Salus Pharma copies	IND	
5/1/2003	Corus	FDA	General correspondence re: Agency feedback on NonClinical plan, 1571	IND 0001	1 of 1
5/5/2003	Corus	FDA	Outline of PK Bioequivalence study attached, 001 amended IND submission	IND 0002	1 of 1
5/12/2003	FDA	Corus	Division review team will meet tomorrow concerning IND, cannot comment on status of IND until after 12:00 meeting	IND	
5/13/2003	FDA	Corus	FDA project manager stated no major issues on IND, but a few questions to be answered before allowing Corus to proceed, telephone contact log	IND	
5/13/2003	Corus	FDA	Outline of PK Bioequivalence study attached, 001 amended IND submission	IND 0003	1 of 1

Cayston IND and NDA Submissions and Correspondence

Date	Doc From	Doc To	Doc Description	Submission and/or Serial Number	Vol.
5/14/2003	FDA	Corus	Corus will be receiving IND comments back from Division later this week, after receipt of letter Corus can proceed	IND	
5/16/2003	FDA	Corus	IND comments and requests for additional information, re: clinical, microbiology biopharmaceutics, pharm-tox and chemistry	IND	
5/16/2003	FDA	Corus	Comments and requests for additonal information re: IND and amendments 001-3	IND	
6/27/2003	Corus	FDA	Response to request for clinical information, Protocol CP-AI-003, investigator information for CP-AI-002 (sent to agency as 003-should have been 004)	IND 0004	1 of 1
7/31/2003	Corus	FDA	Additional investogators for CP-AI-002	IND 0005	1 of 1
8/1/2003	Corus	FDA	Status FDA's review of CP-AI-003, assignmen of FDA staff to project (telephone contact)	IND	
8/12/2003	FDA	Corus	Email re: timing of FDA protocol review	IND	
8/13/2003	Corus	FDA	Delay of draft pharm/tox reports	IND 0007	1 of 1
8/13/2003	Corus	FDA	Correcting misnumbered submissions	IND 0006	1 of 1
8/18/2003	FDA	Corus	Comments on protocol CP-AI-003	IND	
8/18/2003	FDA	Corus	CP-AI-003	IND	
9/4/2003	Corus	FDA	Additional PI information for CP-AI-002	IND 0008	1 of 1
9/23/2003	Corus	FDA	Attachments 8.5, Acute Eye Irritatitation, 8.6 -Acute Dermal Irritation 8.7-Airway Obstruction, 8.8-Comparative Metabolism	IND 0009	5 of 6
9/23/2003	Corus	FDA	Attachment 8.9-Validation of Analytical Method Determination of Azt in Rat and Dog Plasma and Dog Urine	IND 0009	6 of 6
9/23/2003	Corus	FDA	Attachment 8.4, 28 Day Inhalation Tox Study of Azt in Dogs with 14 Day Recovery Period	IND 0009	4 of 6
9/23/2003	Corus	FDA	Attachment 8.3, 28 Day Inhalation Tox Study of Azt in Rats with 14 Day Recovery	IND 0009	3 of 6
9/23/2003	Corus	FDA	Revised Section 8, letter, 1571, Summary of Changes	IND 0009	1 of 6
9/23/2003	Corus	FDA	Attachment 8.1, Single Dose Inhalation Toxicity Study of Azt in Beagle Dogs; 8.2-7 Day Dose Range Finding Tox Study of Azt in Rats	IND 0009	2 of 6

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9/30/2003	Corus	FDA	Additional PI added to Phase 1B, CP-AI-002	IND 0010	1 of 1
10/28/2003	Corus	FDA	Sub investigator being added to U of NC site	IND 0011	1 of 1
11/6/2003	Corus	FDA	FDA response letter (originally dated 8/18/03)	IND 0012	1 of 1
11/21/2003	Corus	FDA	Additional investigators added to study	IND 0013	1 of 1
12/22/2003	Corus	FDA	Principal investigators added to study	IND 0014	1 of 1
1/3/2004	Corus	FDA	Out of office info	IND	
1/22/2004	Corus	FDA	Notification to FDA of Name Change Salus-Corus, Additional investigators added to study	IND 0015	1 of 1
1/29/2004	Corus	FDA	Telephone contact	IND	
2/12/2004	FDA	Corus	From FDA acknowledging company name change from Salus to Corus	IND	
2/13/2004	Corus	FDA	Response to FDA request re: specific questions	IND 00016	1 of 1
2/25/2004	Corus	FDA	Additional investigators added to study	IND 0017	1 of 1
3/12/2004	FDA	Corus	FDA fax reponse to questions dated 2/13/04	IND	
3/25/2004	Corus	FDA	Investigator added to study	IND 0018	1 of 1
3/25/2004	Corus	FDA	Telecon request to discuss phase 3 for AI	IND 0019	1 of 1
4/6/2004	Corus	FDA	Briefing document for telecon 04/29, modify indication, append qol	IND 0020	1 of 1
4/8/2004	FDA	Corus	Letter confirming 4/29/04 telecon meeting	IND	
4/8/2004	FDA	Corus	Letter re: conditions and time of 4/29/04 teleconference	IND	
4/23/2004	Corus	FDA	Sub-investigator added to site	IND 0021	1 of 1
4/27/2004	FDA	Corus	Email Correspondence re: briefing package	IND	
4/27/2004	Corus	FDA	Response to FDA questions for telecon Thurs 4/29/04	IND 0022	1 of 1
4/27/2004	FDA	Corus	Fax from agency with comments re: briefing document	IND	
4/28/2004	Corus	FDA	Response to division request for more information - Quality of life questionnaire	IND	
4/28/2004	FDA	Corus	Email from Susmita Samanta re: CFQ-R	IND	
5/4/2004	Corus	FDA	Request for meeting with agency	IND 0024	1 of 1
5/14/2004	FDA	Corus	FW FDA Meeting date for AI Phase 3	IND	
5/19/2004	FDA	Corus	Letter confirming 7/1/04 telecon meeting	IND	

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5/20/2004	Corus	FDA	FDA/Corus telecon mtg minutes from 4/29/04	IND	
5/25/2004	Corus	FDA	Additional investigator added to study	IND 0025	1 of 1
5/27/2004	FDA	Corus	FDA Meeting Minutes for April 29, 2004	IND	
5/28/2004	Corus	FDA	Qs re: electronic submission	IND 0027	1 of 1
5/28/2004	Corus	FDA	Briefing Document	IND 0026	1 of 1
6/11/2004	Corus	FDA	"Quality of Life" submission	IND 0028	1 of 1
6/25/2004	Corus	FDA	Additional sub-investigators for study	IND 0029	1 of 1
6/28/2004	Corus	FDA	Slide presentation	IND 0030	1 of 1
6/28/2004	Corus	FDA	Annual Report (AI)	IND 0031	1 of 2
6/28/2004	Corus	FDA	References	IND 0031	2 of 2
6/29/2004	FDA	Corus	Email from Agengy re: comments on briefing doc	IND	
6/29/2004	Corus	FDA	Preliminary response to Division's comments re:ph3 study design	IND	
6/29/2004	Corus	FDA	Response to comments on ph 3 study design	IND	
6/29/2004	Corus	FDA	Special Protocol Assessment	IND 0032	1 of 1
7/16/2004	Corus	FDA	Telephone contact	IND	
7/23/2004	Corus	FDA	Email with Draft Meeting Notes	IND	
7/23/2004	Corus	FDA	Add'l Investigators	IND 0033	1 of 1
7/29/2004	FDA	Corus	July 1, 2004 mtg mins from FDA re: indication and ph 3 design	IND	
7/30/2004	FDA	Corus	Email Correspondence from FDA to M.Yeager re: Orphan Drug	IND	
7/30/2004	FDA	Corus	Email with Susmita re telecon 8/24/04, includes original request	IND	
8/13/2004	Corus	FDA	Carcinogenicity Study - 90 Day Rat	IND 0034	4 of 4
8/13/2004	Corus	FDA	Carcinogenicity Study - 90 Day Rat	IND 0034	2 of 4
8/13/2004	Corus	FDA	Carcinogenicity Study - 90 Day Rat	IND 0034	3 of 4
8/13/2004	Corus	FDA	Carcinogenicity Study - 90 Day Rat	IND 0034	1 of 4
8/17/2004	Corus	FDA	Email Correspondence from M. Yeager to FDA re: teleconference and fast track des	IND	
8/17/2004	FDA	Corus	Email Correspondence from FDA to M.Yeager re: CF patient outcomes	IND	
8/18/2004	Corus	FDA	Response to FDA's comments forthcoming (comments included)	IND	

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8/19/2004	Corus	FDA	Fast Track Designation "summary/background info included"	IND 0035	1 of 1
8/19/2004	Corus	FDA	Response to Request for QOL Info	IND 0036	1 of 1
8/25/2004	Corus	FDA	Investigator/Site added	IND 0037	1 of 1
8/31/2004	Corus	FDA	Development Program, telecon notes, 72404 (Corus version to FDA)	IND 0038	1 of 1
9/3/2004	Corus	FDA	Submit Trade name anytime	IND	
9/7/2004	FDA	Corus	Teleconference set up for 9/13/04	IND	
9/9/2004	Corus	FDA	Teleconference Info scheduled 9/13/04 between Corus and FDA	IND 0039	1 of 1
9/10/2004	Corus	FDA	Teleconference 9/13/04, call in number and particpants	IND	
9/13/2004	FDA	Corus	FDA response granting type B meeting/teleconference	IND	
9/13/2004	FDA	Corus	FDA Response to request for QOL teleconference	IND	
9/16/2004	FDA	Corus	Acknowledgement letter for receipt of IND034, special carcinogenicity protocol assessment	IND	
9/16/2004	FDA	Corus	Letter acknowledging receipt of Serial 034	IND	
9/24/2004	Corus	FDA	MCID Study and Teleconference Meeting Minutes from 9/13/04	IND 0040	2 of 2
9/24/2004	Corus	FDA	MCID Study and Teleconference Meeting Minutes from 9/13/04	IND 0040	1 of 2
9/28/2004	Corus	FDA	Email contact re: formal request for end of Phase 2 meeting	IND	
9/30/2004	FDA	Corus	Response to submission 034 (Carcinogenicty)	IND	
10/1/2004	FDA	Corus	Letter from FDA re:034 (original letter)	IND	
10/8/2004	FDA	Corus	Fast track granted	IND	
10/12/2004	FDA	Corus	FDA official meeting mins from 91304 telecon	IND	
10/12/2004	FDA	Corus	End of phase II mtg wFDA confirmed	IND	
10/26/2004	Corus	FDA	End of Phase II Briefing Document	IND 0041	1 of 1
11/10/2004	Corus	FDA	Fax cover page, Serial 042	IND 0041	
11/10/2004	Corus	FDA	Email re: incorrect table and figure included in briefing document, replacement page to follow	IND	
11/12/2004	Corus	FDA	Corrected page (P-29) for End of phase II Mtg	IND 0042	1 of 1
11/17/2004	Corus	FDA	Documentation for Upcoming FDA Face-to-Face Meeting (11/23/2004)	IND 0044	1 of 1
11/17/2004	Corus	FDA	Request for Agency Review of Proposed Trade Name-Cayston	IND 0043	1 of 1

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11/22/2004	Corus	FDA	Requested information for meeting with Agency, Tuesday, 11/23/2004	IND 0044 (duplicate)	1 of 1
12/10/2004	Corus	FDA	Requesting confirmation that tox program is adequate to support approval	IND 0046	4 of 4
12/10/2004	Corus	FDA	Requesting confirmation that tox program is adequate to support approval	IND 0046	3 of 4
12/10/2004	Corus	FDA	Requesting confirmation that tox program is adequate to support approval	IND 0046	2 of 4
12/10/2004	Corus	FDA	Requesting confirmation that tox program is adequate to support approval	IND 0046	1 of 4
12/17/2004	Corus	FDA	Request for Special Protocol Assessment; also includes: meeting minutes, request for telcon, clinical protocols, and documents	IND 0047	1 of 1
12/22/2004	FDA	Corus	Agency meeting minutes from face-to-face 11/23/04	IND	
1/5/2005	Corus		"Note to file" explaining contents of submission 048	IND	
1/5/2005	Corus	FDA	Response to Agency request for additonal copies of submission, serial 047, 1/05/05	IND 0048	1 of 1
1/11/2005	Corus	FDA	request for type B CMC meeting with Agency inform them of end of phase 2	IND 0049	1 of 1
1/21/2005	Corus	FDA	Telephone contact re FDA comments on CP-AI-005	IND	
1/27/2005	FDA	Corus	FDA letter granting request for end of Phase II CMC meeting	IND	
2/3/2005	FDA	Corus	FDA Letter re: Special Protocol Assessment (Serial 047)	IND	
2/4/2005	Corus	FDA	Investigators added to the study	IND 0050	1 of 1
2/11/2005	Corus	FDA	CMC End of Phase 2 brieifng document for meeting 3/08/2005	IND 0051	1 of 1
2/15/2005	Corus	FDA	Email re: submissions and meeting attendee	IND	
2/18/2005	Corus	FDA	Response to Division feedback on CP-AI-005, Amendment to Protocol CP-AI-005, and Submission of Protocol CP-AI-005	IND	
3/2/2005	FDA	Corus	Telephone log of conversation re. Nov 17,2004 submission requesting review of Cayston trade name	IND	
3/2/2005	Corus	FDA	CP-MCID-001, Protocol Amendment	IND 0054	1 of 1
3/4/2005	FDA	Corus	FDA Response to CMC End of Phase 2 Questions	IND	
3/4/2005	Corus	FDA	Investigators Submission	IND 0053	

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3/7/2005	Corus	FDA	CMC End of Phase 2 cancellation request and Corus response to FDA comments	IND	
3/10/2005	Corus	FDA	AI-002 and AI-003 Clinical Study Reports	IND 0055	
3/18/2005	FDA	Corus	Telephone Contact re. AI	IND	
4/4/005	Corus	FDA	Investigator Submission for Protocols CP-MCID-001 and CP-AI-005	IND 0057	1 of 1
4/8/2005	Corus	FDA	Submission of Protocol CP-AI-006	IND 0058	
4/11/2005	Corus	FDA	Email to FDA from Melissa Yeager re. status of open issues	IND	
4/18/2005	Corus	FDA	Proprietary name validtion report for Cayston and Target Product Profile	IND 0056	1 of 1
4/19/2005	Corus	FDA	Corus response to end of phase 2 comments	IND 0059	1 of 1
4/19/2005	Corus	FDA	Email re. FDA response to Corus re/ Corus reply to FDA comments of 005	IND	
5/5/2005	Corus	FDA	Investigator Submission for Protocols CP-MCID-001, CP-AI-005, and CP-AI-007	IND 0060	1 of 1
5/27/2005	FDA	Corus	Email re. status of open issues re. protocol CP-AI-005	IND 0061	1 of 2
6/3/2005	Corus	FDA	Investigator submission for protocols CP-MCID-001, CP-AI-005, CP-AI-007 new, and CP-AI-007 updated	IND 0061	2 of 2
6/3/2005	Corus	FDA	Investigator submission for protocols CP-MCID-001, CP-AI-005, CP-AI-007 new, and CP-AI-07 updated	IND	
6/12/2005	Corus	FDA	Email to FDA re. status of open issues; FDA comments on CP-AI-005, Corus requesting telecomn to discuss preliminary results for	IND	
6/15/2005	Corus	FDA	IND Annual Report	IND 0062	2 of 2
6/15/2005	Corus	FDA	IND Annual Report	IND 0062	1 of 2
6/15/2005	Corus	FDA	Request for meeting and synopsis of protocol CP-AI-004	IND 0063	1 of 1
6/30/2005	Corus	FDA	Telephone contact re: MCID/request for teleconference in Sept 2005	IND	
6/30/2005	FDA	Corus	FDA response to the request for telecon to discuss preliminary CFQ-R results from Ph.2 and Ph.3 for AI, Sept 13, 2005	IND	
7/7/2005	Corus	FDA	Investigator submissions for protocol CP-AI-005 and CP-AI-007	IND 0064	2 of 2
7/7/2005	Corus	FDA	Investigator submissions for Protocol CP-AI-005 and CP-AI-007	IND 0064	1 of 2
8/5/2005	Corus	FDA	Investigator submission for Protocol CP-AI-005, CP-AI-007, CP-AI-006	IND 0065	1 of 1

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8/22/2005	Corus	FDA	Telephone contact: IND 64,402, Cayston-status of issues-protocols, request for feedback	IND	
8/25/2005	Corus	FDA	MCID Interim Results: Studies CP-MCID-001 and CP-AI-005; teleconference, September 13, 2005	IND 0066	1 of 1
8/26/2005	Corus	FDA	Email to FDA re. briefing doc Sept.13 telecon	IND	
8/26/2005	FDA	Corus	Email contact re. IND 64,402 briefing doc Sept.13 telecon	IND	
9/2/2005	Corus	FDA	Corrections to MCID Interim results: Studies CP-MCID-001 and CP-AI-005: Teleconference	IND 0067	1 of 1
9/7/2005	Corus	FDA	Investigator submission for Protocol CP-AI-005, CP-AI-007, CP-AI-006	IND 0068	2 of 2
9/7/2005	Corus	FDA	Investigator submission for Protocol CP-AI-005, CP-AI-007, CP-AI-006	IND 0068	1 of 2
9/8/2005	Corus	FDA	Email to FDA re IND 64,402 teleconference slides with updated information	IND	
9/12/2005	Corus	FDA	Email to FDA with teleconference slides with updated information	IND	
9/21/2005	Corus	FDA	Email to FDA re. meeting notes, re. meeting notes, request for meeting, new address…	IND	
9/22/2005	Corus	FDA	Meeting request; request for biostatistician review; September 13 telecon…	IND 0069	1 of 1
9/22/2005	Corus	FDA	Email to FDA re. IND 64,402; serial no 069 meeting notes…	IND	
9/29/2005	Corus	FDA	CMC Information Amendment	IND 0070	1 of 1
10/5/2005	Corus	FDA	Investigator submission for Protocol CP-AI-005, CP-AI-007, CP-AI-006	IND 0071	1 of 1
10/6/2005	Corus	FDA	Telephone contact re FDA meeting request and meeting minutes	IND	
10/7/2005	FDA	Corus	Letter from FDA confirming schedule for November 29, 2005…	IND	
10/7/2005	Corus	FDA	Telephone contact re. FDA meeting meeting date and time	IND	
10/13/2005	FDA	Corus	Letter from FDA re official minutes from September 13, 2005 teleconference	IND	
10/13/2005	Corus	FDA	Serial 069 Meeting notes and other issues	IND	
10/26/2005	Corus	FDA	Email to FDA re. Questions for Dr. Scott	IND	
10/28/2005	Corus	FDA	Email correspondence to FDA with electronic submission Serial 072 attached	IND	1 of 2
10/28/2005	Corus	FDA	Email correspondence to FDA with electronic submission Serial 072 attached	IND	1 & 2 of 2

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10/28/2005	Corus	FDA	Briefing document for November 28, 2005 meeting...	IND 0072	1 of 2
10/30/2005	Corus	FDA	Email to FDA requesting correct Silver Spring address	IND	
10/31/2005	FDA	Corus	Email to Corus re correct zip code for Silver Sprind address	IND	
11/2/2005	Corus	FDA	Investigator Submission for Protocol CP-AI-005, CP-AI-007, CP-AI-006…	IND 0073	1 of 1
11/2/2005	Corus	FDA	Email to FDA re. arrival of documents at Silver Spring address	IND	
11/17/2005	Corus	FDA	Email to FDA re Logistics for Nov. 29th meeting	IND	
11/21/2005	FDA	Corus	Email from FDA re Logistics for Nov. 29th meeting	IND	
11/21/2005	Corus	FDA	Email to FDA with list of attendees for Nov. 29th meeting	IND	
11/22/2005	Corus	FDA	Corus' questions for face to face meeting with FDA	IND	
11/28/2005	Corus	FDA	Logistics for Nov 29th meeting	IND	
12/7/2005	Corus	FDA	Investigator Submission for Protocol CP-AI-007, and CP-AI-006	IND 0074	1 of 1
12/8/2005	Corus	FDA	Status of notes; request for discusscussions with biostatistician	IND	
12/10/2005	Corus	FDA	Notes for Nov 29 Meeting	IND	
12/13/2005	Corus	FDA	Questions for Biostatistics Review	IND	
12/16/2005	Corus	FDA	Notes for November 29, 2005 Divisision Meeting; Questions and Information…	IND 0075	1 of 1
12/21/2005	Corus	FDA	CMC Information Amendment	IND 0076	1 of 1
1/5/2006	FDA	Corus	Questions for Biostatistician Reviewers Regarding the CP-AI-007 Sample…	IND	
1/6/2006	Corus	FDA	Investigator Submission for Protocols CP-AI-005, CP-AI-007, and CP-AI-006	IND 0077	1 of 1
1/9/2006	Corus	FDA	Corus Pharma's response to Division comments; revised…	IND 0078	1 of 1
1/18/2006	FDA	Corus	Email from FDA re: Corus Pharma Aztreonam Lysince for Inhalation eCTD	IND	
2/8/2006	FDA	Corus	Official Minutes from Nov 29, 2005 Meeting…	IND	
2/8/2006	Corus	FDA	Investigator Submission for Protocol CP-AI-007, and CP-AI-006	IND 0079	1 of 1
3/10/2006	Corus	FDA	Investigator Submission for Protocol CP-AI-005, CP-AI-007, and CP-AI-006	IND 0080	1 of 1
3/16/2006	Corus	FDA	Briefing document, response to division request for additional MCID…	IND 0081	1 of 1
4/6/2006	Corus	FDA	Investigator Submission for Protocol CP-AI-007, and CP-AI-006	IND 0082	1 of 1

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4/13/2006	Corus	FDA	Email contact informing the FDA of the upcoming Request for Submission…	IND	
4/17/2006	Corus	FDA	Request for Submission of Portions of an Application	IND 0085	1 of 1
4/18/2006	Corus	FDA	Corus Pharma's Request for a Type C Meeting	IND 0086	1 of 1
5/3/2006	FDA	Corus	Letter confirming July 11, 2006 meeting with FDA	IND	
5/3/2006	Corus	FDA	Request for NDA number for Aztreonam Lysine for Inhalation	IND	
5/3/2006	Corus	FDA	AI, Meeting and NDA Information	IND	
5/4/2006	FDA	Corus	Statistical Comments_IND 64402_224.doc	IND	
5/5/2006	Corus	FDA	Investigator Submission for Protocol CP-AI-005, CP-AI-007, and CP-AI-006…	IND 0087	1 of 1
5/12/2006	Corus	FDA	CP-AI-007 Statistical Review Com...	IND 0088	1 of 1
6/6/2006	Corus	FDA	Submission of Amended Protocol CP-AI-007	IND 0090	1 of 1
6/6/2006	Corus	FDA	Investigator Submission for Protocols CP-AI-007 and CP-AI-006	IND 0089	1 of 1
6/8/2006	Corus	FDA	IND Annual Report	IND 0091	1 of 1
6/9/2006	Corus	FDA	Briefing Document for July 11, 2006 Meeting	IND 0092	1 of 1
6/13/2006	Corus	FDA	Telephone Contact: Questions Regarding Rolling Review Proposal	IND	
6/13/2006	Corus	FDA	Telephone Contact: Aztreonam for Inhalation…	IND	
6/19/2006	Corus	FDA	Telephone Contact: Orphan Drug Application Fee Waiver…	IND	
6/19/2006	Corus	FDA	Telephone Contact: CMC Comments on Briefing Package...	IND	
6/28/2006	FDA	Corus	Plan for step-wise submission of sections...	IND	
7/6/2006	Corus	FDA	Investigator Submission for Protocol CP-AI-007 and CP-AI-006	IND 0093	1 of 1
7/7/2006	FDA	Gilead	Comments on your July 11 Meeting Package	IND	
7/14/2006	Corus	FDA	Telephone contact log w/Ken Edmunds about eCTD	IND	
7/14/2006	Corus	FDA	Initial eCTD/NDA (NonClinical module) signed cover letter, forms, (cd download	NDA 0000	
7/18/2006	Corus	FDA	Meeting Minutes; Out of Office Contacts...	IND	1 of 1
7/19/2006	Corus	FDA	Notes for July 11, 2006 Division Meeting	IND 0095	
8/4/2006	FDA	Corus	Official Minutes of the July 11, 2006 Division Meeting	IND	1 of 1

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8/8/2006	Corus	FDA	Country Specific Protocols for CP-AI-006	IND 0097	1 of 1
8/10/2006	Corus	FDA	Omitted Serial Number	IND 0098	1 of 1
8/11/2006	Corus	FDA	Response to Statistical Comments	IND 0099	
8/23/2006	Gilead	FDA	Notification of Company Acquisition and Name Change to …	IND	1 of 1
8/23/2006	Gilead	FDA	Notification of Company Acquisition…	IND 0100	1 of 1
8/23/2006	Gilead	FDA	Carcinogenicity Study Update, Request for Guidance	IND 0101	1 of 1
8/28/2006	FDA	Gilead	FDA Contact, eCTD & change of company	NDA	
9/5/2006	Gilead	FDA	Submission of Amended Protocol CP-AI-007, New Version…	IND 0102	1 of 1
9/6/2006	Corus	FDA	Investigator Submission for Protocols CP-AI-007, CP-AI-006	IND 0103	1 of 1
9/17/2006	FDA	Gilead	FDA contact, Carc study misc. questions for project...	IND	
9/21/2006	Gilead	FDA	Telephone contact: Peggy H. assigning new NDA # 050-814	NDA	
9/22/2006	Gilead	FDA	Telephone contact: obtaining new NDA number	NDA	
10/13/2006	Gilead	FDA	Investigator submission for CP-AI-007, CP-AI-006	IND 0104	1 of 1
10/20/2006	Gilead	FDA	Request for a Pre-NDA, Type B Meeting	IND 0105	1 of 1
10/30/2006	Gilead	FDA	IND Safety Report	IND 0106	1 of 1
11/6/2006	FDA	Gilead	FDA response to meeting request	IND	
11/17/2006	Gilead	FDA	Investigator Submission for Protocols CP-AI-007 and CP-AI-006	IND 0107	1 of 1
11/29/2006	Gilead	FDA	Sensitivity Analyses for Primary Endpoint	IND 0108	1 of 1
12/5/2006	Gilead	FDA	Carcinogenicity Study, NDA 50-814/IND 64,402	IND	
12/5/2006	FDA	Gilead	Carcinogenicity Study	IND	
12/6/2006	Gilead	FDA	Carcinogenicity Study, NDA 50-814/IND 64,402	IND	
12/6/2006	FDA	Gilead	Carcinogenicity Study, NDA 50-814/IND 64,402	IND	
12/8/2006	FDA	Gilead	RE: NDA 50-814/IND 64,402	IND	
12/13/2006	Gilead	FDA	Investigator Submission for Protoco;s CP-AI-007 & CP-AI-006	IND 0109	1 of 1
12/21/2006	Gilead	FDA	Progress Report on Study CP-AI-005	IND	
1/12/2007	Gilead	FDA	Pre-NDA Meeting Briefing Information	IND 0110	1 of 4

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2/12/2009	Gilead	FDA	NDA 50-814 dispute resolution status	NDA	
2/13/2009	FDA	Gilead	EMAIL-AZLI dispute resolution status	NDA	
2/18/2009	Gilead	FDA	EMAIL-AZLI dispute resolution status	NDA	
2/18/2009	FDA	Gilead	EMAIL-AZLI dispute resolution status	NDA	
2/18/2009	Gilead	FDA	EMAIL-AZLI dispute resolution status	NDA	
2/18/2009	FDA	Gilead	EMAIL-AZLI dispute resolution status (attachment)	NDA	
2/18/2009	FDA	Gilead	LTR-FDA response on Aztreonam	NDA	
2/18/2009	FDA	Gilead	LTR-Response to formal dispute resolution	NDA	
2/26/2009	FDA	Gilead	EMAIL-Type B Meeting	NDA	
3/2/2009	Gilead	FDA	EMAIL-Dates for Type B Meeting	NDA	
3/5/2009	Gilead	FDA	CR-Amy Bertha, Dispute Resolution Manager	NDA	
3/5/2009	Gilead	FDA	EMAIL- Type B Meeting	NDA	
3/5/2009	FDA	Gilead	EMAIL- Type B Meeting	NDA	
3/5/2009	Gilead	FDA	EMAIL-Type B Meeting date confirmed	NDA	
3/5/2009	Gilead	FDA	EMAIL-Type B Meeting MP submit date	NDA	
3/13/2009	Gilead	FDA	Formal Dispute Resolution Request	NDA 0032	
3/16/2009	FDA	Gilead	LTR-Use of AZLI Diluent	NDA	
3/16/2009	FDA	Gilead	LTR-Use of AZLI Diluent	IND	1 of 1
3/18/2009	Gilead	FDA	CR-AZLI Dispute Resolution	NDA	
3/23/2009	Gilead	FDA	CR-Type B Meeting Package	NDA	
3/23/2009	FDA	Gilead	EMAIL-MP for F2F Meeting	NDA	
3/24/2009	FDA	Gilead	EMAIL-MP for F2F Meeting	NDA	
3/25/2009	Gilead	FDA	CR-Type B Meeting	NDA	
3/30/2009	FDA	Gilead	EMAIL-New meeting date	NDA	
4/3/2009	Gilead	FDA	Type B Meeting Background Materials	NDA 0033	
4/3/2009	Gilead	FDA	EMAIL-MP for May 15 Meeting	NDA	
4/3/2009	Gilead	FDA	EMAIL-Type B Meeting Discussion Items	NDA	
4/6/2009	Gilead	FDA	EMAIL-MP for F2F Meeting	NDA	

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4/7/2009	FDA	Gilead	CR-Bertha-FDRR Question.	NDA	
4/7/2009	Gilead	FDA	EMAIL-MP for F2F Meeting	NDA	
4/8/2009	Gilead	FDA	Type B Meeting Background Materials, Resubmission of Cover Letter	NDA 0034	
4/8/2009	Gilead	FDA	EMAIL-FDRR Additional Information Request	NDA	
4/8/2009	Gilead	FDA	EMAIL-FDRR Additional Information Request	NDA	
4/8/2009	FDA	Gilead	EMAIL-MP for F2F Meeting	NDA	
4/8/2009	Gilead	FDA	EMAIL-MP for F2F Meeting	NDA	
4/8/2009	Gilead	FDA	Response to request for information	NDA 0035	
4/10/2009	FDA	Gilead	CR-Quaintance-FDRR Meeting	NDA	
4/13/2009	Gilead	FDA	CR-Bertha-FDRR Meeting	NDA	
4/14/2009	FDA	Gilead	EMAIL-AZLI NDA FDRR-attached letter	NDA	
4/15/2009	FDA	Gilead	04 15-EMAIL-AZLI NDA 050814 FDRR	NDA	
4/15/2009	Gilead	FDA	EMAIL-AZLI NDA FDRR	NDA	
4/16/2009	Gilead	FDA	EMAIL-AZLI Bronchiectasis Fast Track Request	IND	1 of 1
4/17/2009	FDA	Gilead	LTR-Response - Formal dispute resolution Request	NDA	
4/20/2009	Gilead	FDA	EMAIL-AZLI NDA 050814 FDRR	NDA	
4/20/2009	Gilead	FDA	EMAIL-AZLI NDA FDRR	NDA	
4/22/2009	Gilead	FDA	Formal Dispute Resolution Meeting Background Materials	NDA 0036	
4/23/2009	Gilead	FDA	EMAIL-AZLI NDA 050814 FDRR	NDA	
4/23/2009	FDA	Gilead	EMAIL-AZLI NDA 050814 FDRR	NDA	
4/23/2009	FDA	Gilead	EMAIL-AZLI NDA 050814 FDRR	NDA	
4/24/2009	FDA	Gilead	EMAIL-Gilead FDRR-alternate contact	NDA	
4/24/2009	Gilead	FDA	EMAIL-Slides presented at April 24 meeting, AZLI FDRR	NDA	
4/24/2009	FDA	Gilead	EMAIL-Slides presented at April 24 meeting, AZLI FDRR	NDA	
4/24/2009	Gilead	FDA	EMAIL-FDA Form 1572 for GS-US-205-0110	IND	
4/27/2009	Gilead	FDA	IND Safety Report, Mfr. Report #2009-0021430 (haemoptysis)	IND 0196	1 of 1
4/29/2004	FDA	Gilead	CR-Hyon-May 15 Type B Mtg	NDA	
4/29/2004	Gilead	FDA	EMAIL-AZLI FDRR April 24 Meeting Followup	NDA	

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4/29/2004	FDA	Gilead	EMAIL-AZLI FDRR April 24 Meeting Followup	NDA	
4/29/2004	FDA	Gilead	EMAIL-AZLI NDA 050814 - Type B Meeting on 15 May 2009	NDA	
4/29/2004	Gilead	FDA	EMAIL-AZLI NDA 050814 - Type B Meeting on May 15 2009	NDA	
5/1/2009	Gilead	FDA	IND Safety Report, Mfr. Report #2009-0021430 (haemoptysis)	IND 0197	1 of 1
5/5/2009	FDA	Gilead	CR-Hyon-May 15 Type B Mtg	NDA	
5/5/2009	Gilead	FDA	EMAIL-May 15 Type B Meeting, AZLI NDA 050814-51448	NDA	
5/5/2009	Gilead	FDA	EMAIL-May 15 Type B Meeting AZLI NDA 050814-51448	NDA	
5/8/2009	Gilead	FDA	Formal Dispute Resolution Meeting Follow-up Items	NDA 0037	
5/8/2009	Gilead	FDA	EMAIL-Response to request for info AZLI FDRR April 24 mtg	NDA	
5/12/2009	FDA	Gilead	EMAIL-Form 1572 for Protocol GS-US-205-0110	IND	
5/12/2009	FDA	Gilead	EMAIL-Form 1572 for Protocol GS-US-205-0110	IND	
5/13/2009	FDA	Gilead	CR-Quaintance-May 15 Type B Meeting	NDA	
5/13/2009	Gilead	FDA	EMAIL-May 15 Type B Meeting-51424	NDA	
5/13/2009	Gilead	FDA	EMAIL-May 15 Type B Meeting with DAIOP	NDA	
5/13/2009	FDA	Gilead	EMAIL-May 15 Type B Meeting with DAIOP	NDA	
5/14/2009	FDA	Gilead	EMAIL-May 15 Type B Meeting	NDA	
5/15/2009	Gilead	FDA	Cancellation of May 15, 2009 Type B Meeting, Change in Sponsor Contact	NDA 0038	
5/15/2009	Gilead	FDA	EMAIL-AZLI NDA 050814 FDRR	NDA	
5/15/2009	FDA	Gilead	EMAIL-May 15 2009 Type B Mtg	NDA	
5/15/2009	Gilead	FDA	EMAIL-May 15 Type B Meeting Cancellation Request-	NDA	
5/15/2009	FDA	Gilead	EMAIL-IND64402 Protocol copies AIRCF1 AIRCF2	IND	
5/15/2009	Gilead	FDA	EMAIL-IND64402 Protocol copies AIRCF1 AIRCF2	IND	
5/16/2009	Gilead	FDA	Protocol Amend - New Invest	IND 0198	1 of 1
5/19/2009	Gilead	FDA	CR-Quiantance-FDRR2	NDA	
5/21/2009	Gilead	FDA	EMAIL-Response to Request by Dr ONeill AZLI FDRR	NDA	
5/21/2009	Gilead	FDA	Reponse to Request for Information from the April 24, 2009 Meeting	NDA 0039	
5/22/2009	Gilead	FDA	CR-Bertha_FDRR Response Delay	NDA	
5/28/2009	FDA	Gilead	EMAIL-Update on AZLI FDRR Status	NDA	

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5/28/2009	Gilead	FDA	EMAIL-Update on AZLI FDRR Status	NDA	
6/10/2006	Gilead	FDA	Protocol Amend - New Invest…	IND 0199	1 of 1
6/11/2009	Gilead	FDA	Annual Report	IND 0200	
6/19/2009	Gilead	FDA	Protocol Amend - Updated Invest…	IND 0201	
6/15/2009	Gilead	FDA	CR-Bertha-FDRR Response from Kweder	NDA	
6/17/2009	FDA	Gilead	EMAIL- FDRR - response and meeting minutes	NDA	
6/18/2009	FDA	Gilead	CR-Hyon-OND FDRR Response and Meeting with DAIOP	NDA	
6/18/2009	FDA	Gilead	CR-Hyon-Response from (OND) regarding formal dispute resolution	NDA	
6/23/2009	FDA	Gilead	LTR-Response for Formal Dispute Resolution	NDA	
6/24/2009	Gilead	FDA	EMAIL - DOAIDP - Wiley Chambers	NDA	
6/24/2009	Gilead	FDA	General correspondence Additional Sponsor Contact	IND 0202	
6/29/2009	FDA	Gilead	CR-Hyon-Appeal formal dispute resolution	NDA	
6/29/2009	FDA	Gilead	CR-Hyon-CRL Response and Meeting with DAIOP	NDA	
6/29/2009	FDA	Gilead	LTR-Official minutes for 24APR2009 Formal Dispute Resolution mtg	NDA	
6/30/2009	FDA	Gilead	EMAIL-Correspondence	NDA	
7/9/2009	FDA	FDA	Protocol Amend - New Invest…	IND 0203	
7/14/2009	Gilead	FDA	IND Safety Report, MFR. Report #2009-0022821…	IND 0204	
7/19/2009	FDA	Gilead	EMAIL-Correspondence From FDA	NDA	
7/19/2009	Gilead	FDA	EMAIL-Correspondence to FDA	NDA	
7/30/2009	Gilead	FDA	IND Safety Report, MFR. Report #2009-0022821…	IND 0205	
7/30/2009	Gilead	FDA	IND Safety Report, Mfr. Report #2009-0023129...	IND 0206	
8/5/2009	Gilead	FDA	Protocol Amend - Change in Protocol…	IND 0207	
8/11/2009	Gilead	FDA	EMAIL-Safety reporting follow-up nebulizer	IND	
8/11/2009	Gilead	FDA	Protocol Amend - New Invest…	IND 0208	
8/11/2009	Gilead	FDA	Resubmission	NDA 0040	
8/12/2009	Gilead	FDA	EMAIL-Resubmission	NDA	
8/19/2009	FDA	Gilead	CR-Hyon-Labeling in Resubmission	NDA	
8/21/2009	Gilead	FDA	EMAIL-Labeling Submission to AZLI NDA 050814	NDA	

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8/21/2009	FDA	Gilead	EMAIL-Labeling Submission to AZLI NDA 050814 response	NDA	
8/21/2009	Gilead	FDA	Submission of Proposed Labeling Text	NDA 0041	
8/25/2009	FDA	Gilead	EMAIL-CMC IR for NDA 50-814	NDA	
8/25/2009	Gilead	FDA	IND Safety Report, Mfr Report #2009-0022821…	IND 0209	
8/26/2009	FDA	Gilead	CR-Hyon-Tox Rpt to Resubmission	NDA	
8/26/2009	Gilead	FDA	Submission of Updated Manufacturing Facilities and Contact Information	NDA 0042	
8/26/2009	FDA	Gilead	EMAIL-Resubmission Acknowledgement Letter	NDA	
8/26/2009	FDA	Gilead	EMAIL-Statistical IR for NDA 50-814	NDA	
8/26/2009	FDA	Gilead	LTR-Resubmission Acknowledgement Letter	NDA	
8/31/2009	FDA	Gilead	LTR-December Anti-Infective Drugs Advisory Committee Mtg	NDA	
8/31/2009	FDA	Gilead	LTR-Receipt acknowledgement of Aug 11 resubmission	NDA	
9/4/2009	Gilead	FDA	Protocol Amendment: New Protocol	IND 0210	
9/9/2009	FDA	Gilead	EMAIL-December Mtg Anti-Infective Drugs Advisory Committee	NDA	
9/9/2009	FDA	Gilead	Protocol Amendment: New and Updated Invest…	IND 0211	
9/11/2009	Gilead	FDA	Response to Request for Additional Information	NDA 0043	
9/14/2009	Gilead	FDA	EMAIL-26Aug09 Statistical IR for NDA 50-814	NDA	
9/15/2009	FDA	Gilead	EMAIL-December Meeting of the AIDAC	NDA	
9/15/2009	FDA	Gilead	EMAIL-December Meeting of the Anti-Infective Drugs Advisory Committee	NDA	
9/15/2009	Gilead	FDA	IND Safety Report, Mfr Report # 2009-0022821...	IND 0212	
9/18/2009	FDA	Gilead	IR for Toxicology Study Reports	NDA	
9/21/2009	FDA	Gilead	EMAIL-14Sept09 Statistical IR for NDA 50-814	NDA	
9/21/2009	Gilead	FDA	EMAIL-December Meeting AIDAC - draft sponsor attendee list	NDA	
9/22/2009	Gilead	FDA	Response to Request for Final Toxicology Study Report	NDA 0044	
9/22/2009	FDA	Gilead	EMAIL-December Meeting AIDAC-timeline	NDA	
9/23/2009	Gilead	FDA	EMAIL-18Sept09 IR for Toxicology Study Reports	NDA	
9/25/2009	Gilead	FDA	EMAIL-December Meeting of the AIDAC-list of investigators	NDA	
9/28/2009	FDA	Gilead	EMAIL-December Meeting of the AIDAC-list of investigators	NDA	
10/5/2009	FDA	Gilead	EMAIL- Type A meeting request	IND	

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10/9/2009	FDA	Gilead	EMAIL- Type A meeting request, AZLI IND 64,402	IND	
10/12/2009	Gilead	FDA	Protocol Amendment: New and Updated Invest…	IND 0213	
10/13/2009	Gilead	FDA	Protocol Amendment: Change in Protocol	IND 0214	
10/14/2009	Gilead	FDA	EMAIL-14Sept09 Statistical IR for NDA 50-814	NDA	
10/15/2009	Gilead	FDA	Response to Request for Additional Analyses and Safety Dataset	NDA 0045	
10/15/2009	Gilead	FDA	EMAIL-Type A meeting request, AZLI IND 64,402	IND	
10/15/2009	Gilead	FDA	Request for a Type A meeting and Background…	IND 0215	
10/19/2009	FDA	Gilead	EMAIL-Type A meeting request, AZLI IND 64,402-Correspondence…	IND	
10/19/2009	FDA	Gilead	EMAIL-Type A meeting request, AZLI IND 64,402-Correspondence…	IND	
10/19/2009	FDA	Gilead	EMAIL-Type A meeting request, AZLI IND 64,402-Correspondence…	IND	
10/23/2009	FDA	Gilead	EMAIL-Type A meeting request, AZLI IND 64,402-Copy of Meeting…	IND	
10/23/2009	FDA	Gilead	LTR-Type A Meeting Granted	IND	
10/26/2009	FDA	Gilead	EMAIL-FR Notice for December 10th Anti-Infective Drugs Advisory Committee Meeting	NDA	
10/29/2009	FDA	Gilead	EMAIL-Type A meeting request, AZLI IND 64,402-Correspondence…	IND	
10/29/2009	Gilead	FDA	EMAIL-Type A meeting request, AZLI IND 64,402-Correspondence…	IND	
10/30/2009	Gilead	FDA	Preliminary Results of Study GS-US-205-0117	NDA 0046	
10/30/2009	Gilead	FDA	Letter of Cross-Reference	IND 0216	
11/2/2009	FDA	Gilead	LTR-Type A Meeting Granted	IND	
11/5/2009	FDA	Gilead	EMAIL-Backgrounders Received for December 10, 2009 Anti-Infective Drugs Advisory Committee ...	NDA	
11/5/2009	Gilead	FDA	EMAIL-Backgrounders Received for December 10, 2009 Anti-Infective Drugs Advisory Committee ...	NDA	
11/6/2009	FDA	Gilead	EMAIL-Type A Telecon, IND 64,402	IND	
11/9/2009	Gilead	FDA	Protocol Amendment: New and Updated Invest…	IND 0217	
11/9/2009	Gilead	FDA	Letter of Cross-Reference	IND 0218	
11/9/2009	Gilead	FDA	EMAIL-December 10th Anti-Infective Drugs Advisory Committee Meeting	NDA	
11/10/2009	Gilead	FDA	EMAIL-Gilead attendee list - Nov.10 Type A Telecon…	IND	

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11/10/2009	Gilead	FDA	Efficacy Amendment	NDA 0047	
11/10/2009	FDA	Gilead	EMAIL-December 10th Anti-Infective Drugs Advisory Committee Meeting	NDA	
11/10/2009	Gilead	FDA	EMAIL- December 10th Anti-Infective Drugs Advisory Committee Meeting	NDA	
11/12/2009	FDA	Gilead	EMAIL-November 10, 2009 submission-Correspondence from FDA	NDA	
11/12/2009	Gilead	FDA	EMAIL-November 10, 2009 submission-Correspondence to FDA	NDA	
11/13/2009	Gilead	FDA	Revised 3.2.P.2 Document	NDA 0048	
11/13/2009	Gilead	FDA	EMAIL- December 10th Anti-Infective Drugs Advisory Committee Meeting	NDA	
11/13/2009	Gilead	FDA	EMAIL-November 10, 2009 submission-Correspondence to FDA	NDA	
11/13/2009	FDA	Gilead	LTR-FDA Briefing Document	NDA	
11/13/2009	Gilead	FDA	Protocol Amendment: Change in Protocol	IND 0219	
11/15/2009	FDA	Gilead	EMAIL-November 10, 2009 submission-Correspondence from FDA	NDA	
11/20/2009	Gilead	FDA	EMAIL-December 10th AIDAC Mtg	NDA	
11/20/2009	Gilead	FDA	EMAIL-FR Notice for Dec 10th AIDAC Mtg	NDA	
11/23/2009	FDA	Gilead	EMAIL-December 10th Anti-Infective Drugs Advisory Committee Meeting	NDA	
11/24/2009	FDA	Gilead	EMAIL-December 10th Anti-Infective Drugs Advisory Committee Meeting	NDA	
11/24/2009	FDA	Gilead	LETTER-Advice-Information Request	IND	
11/24/2009	FDA	Gilead	LTR- advice Info Request Clinical Micro Comments	IND	
11/25/2009	Gilead	FDA	Meeting Minutes from Nov.10,2009 Type A Meeting	IND 0220	
11/25/2009	Gilead	FDA	IND Safety Report, Mfr Report #2009-0023129…	IND 0221	
12/2/2009	FDA	Gilead	EMAIL-December 10th Anti-Infective Drugs Advisory Committee Meeting	NDA	
12/3/2009	FDA	Gilead	EMAIL-December 10th Anti-Infective Drugs Advisory Committee Meeting	NDA	
12/3/2009	FDA	Gilead	LTR- advice Info Request Clinical Micro Comments	IND	
12/5/2009	Gilead	FDA	EMAIL- December 10th AIDAC Mtg	NDA	
12/7/2009	FDA	Gilead	EMAIL-December 10th Anti-Infective Drugs Advisory Committee Meeting	NDA	
12/7/2009	FDA	Gilead	LETTER- Nov.10 Type A Telecon	IND	
12/7/2009	FDA	Gilead	LTR-Meeting minutes to discuss H2H SAP	IND	
12/8/2009	Gilead	FDA	IND Safety Report, Mfr Report #2009-0023129…	IND 0222	
12/11/2009	Gilead	FDA	Protocol Amendment: Updated Invest…	IND 0223	

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12/14/2009	FDA	Gilead	LTR-Meeting minutes to discuss H2H SAP	IND	
12/17/2009	Gilead	FDA	GS-US-205-0117 CSR	IND 0224	
12/17/2009	FDA	Gilead	LTR-Discipline Review Letter	NDA	
12/21/2009	Gilead	FDA	Response to FDA Request for Information	IND 0225	
12/30/2009	Gilead	FDA	IND Safety Report, Mfr Report #2009-0026082…	IND 0226	
1/7/2010	Gilead	FDA	EMAIL-Slides from AIDAC Mtg	NDA	
1/12/2010	Gilead	FDA	Protocol Amendment: Updated Invest…	IND 0227	
1/14/2010	FDA	Gilead	EMAIL-Request for Pending NDA 50-814	NDA	
1/18/2010	Gilead	FDA	EMAIL-Request for Pending NDA 50-814	NDA	
1/18/2010	Gilead	FDA	Postmarketing Commitment	NDA 0049	
1/20/2010	Gilead	FDA	CR-LeSane-Labeling Negotiations	NDA	
1/21/2010	Gilead	FDA	Revised Draft Carton and Container Labels	NDA 0050	
1/21/2010	Gilead	FDA	IND Safety Re[prt, Mrf Report #2009-0026082…	IND 0228	
1/22/2010	FDA	Gilead	CR-LeSane-Samples of Vial Label and Diluent Ampule	NDA	
1/25/2010	FDA	Gilead	EMAIL-Proposed DRAFT Labeling for NDA 50-814	NDA	
1/27/2010	Gilead	FDA	LTR-Sample of the Cayston Vial Label	NDA	
1/28/2010	Gilead	FDA	IND Safety Report, Mfr Report #2010-0026467…	IND 0229	
1/29/2010	Gilead	FDA	CR-LeSane-Status of Gilead comments on Label	NDA	
1/29/2010	FDA	Gilead	EMAIL-NDA 50-814 Questions	NDA	
2/1/2010	Gilead	FDA	EMAIL-Altera 510(k) Clearance	NDA	
2/1/2010	Gilead	FDA	EMAIL-Proposed PI and SOC	NDA	
2/3/2010	Gilead	FDA	EMAIL-Diluent Ampule Embossed Text	NDA	
2/3/2010	Gilead	FDA	CR-LeSane-Cayston Labeling Update and Altera 510(k)	NDA	
2/5/2010	FDA	Gilead	EMAIL-Cayston Labeling and PI Edits	NDA	
2/5/2010	Gilead	FDA	EMAIL-Seq 0050 Rationale for diluent text	NDA	
2/5/2010	FDA	Gilead	EMAIL-Version of Word	NDA	
2/5/2010	FDA	Gilead	CR-Chambers-Altera 510(k)	NDA	
2/7/2010	Gilead	FDA	EMAIL-Altera IFU and Carton Text	NDA	

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2/8/2010	Gilead	FDA	Right of Reference	NDA 0051	
2/8/2010	Gilead	FDA	EMAIL-VM Follow-up to Frances L	NDA	
2/9/2010	FDA	Gilead	CR-Chambers - 0800 Urgency of Altera 510(k)	NDA	
2/9/2010	FDA	Gilead	CR-Chambers - 1800 - Status of NDA due to 510(k) delay		
2/9/2010	Gilead	FDA	General Correspondence	NDA 0052	
2/9/2010	Gilead	FDA	EMAIL-Seq 0052 Gen Corres and PPI	NDA	
2/9/2010	Gilead	FDA	EMAIL-Altera IFU and Carton Text	NDA	
2/9/2010	Gilead	FDA	EMAIL-PARI 510(k) re-submission	NDA	
2/9/2010	Gilead	FDA	Protocol Amendment: New and Updated Invest…	IND 0230	
2/10/2010	FDA	Gilead	EMAIL-Edits to proposed Cayston PI	NDA	
2/10/2010	Gilead	FDA	CR-Chambers - Status of the NDA	NDA	
2/10/2010	FDA	Gilead	EMAIL-Labeling Comments	NDA	
2/11/2010	Gilead	FDA	Revised Draft Labeling	NDA 0053	
2/11/2010	Gilead	FDA	EMAIL-Seq 0053 Labeling	NDA	
2/12/2010	FDA	Gilead	CR-Chambers-Update on NDA Action Date	NDA	
2/12/2010	Gilead	FDA	Altera Nebulizer System Labeling	NDA 0054	
2/12/2010	Gilead	FDA	EMAIL- Seq 0054 Altera Nebulizer lebling	NDA	
2/15/2010	Gilead	FDA	EMAIL-Feb 2010 Exp of FWA00006530	IND	
2/16/2010	Gilead	FDA	CR-Update on NDA PDUFA Extension	NDA	
2/18/2010	Gilead	FDA	IND Safety Report, Mfr Report #2010-0026082…	IND 0231	
2/22/2010	FDA	Gilead	EMAIL- Action Letter	NDA	
2/23/2010	Gilead	FDA	SPL for approved NDA 050814, Final Printed Carton and Container Labels	NDA 0055	
2/24/2010	Gilead	FDA	EMAIL-Couresty Copy of Seq 0050	NDA	
2/24/2010	Gilead	FDA	CR-Final Cayston Labeling	NDA	
2/25/2010	Gilead	FDA	IND Safety Report, Mrf Report #2010-0026082…	IND 0232	
2/26/2010	FDA	Gilead	EMAIL-Courtesy Copy of Seq 0055	NDA	
2/28/2010	Gilead	FDA	EMAIL- 0050 Raionale for Diluent Text	NDA	
3/3/2010	Gilead	FDA	EMAIL-SPL Req for Clarity on Cmnts	NDA	

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3/7/2010	FDA	Gilead	EMAIL-SPL Req for Clarity on Cmnts-2	NDA	
3/8/2010	FDA	Gilead	EMAIL-SPL Req for Clarity on Cmnts-2	NDA	
3/10/2010	FDA	Gilead	LTR-NDA Approval	NDA	

EXHIBIT 10

PEDIATRICS®
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Cystic Fibrosis: Comparison of Two Mucolytic Drugs for Inhalation Treatment (Acetylcysteine and Arginine Hydrochloride)

Hans-Joachim Dietzsch, Bodo Gottschalk, Klaus Heyne, Wolfgang Leupold and Peter Wunderlich

Pediatrics 1975;55;96-100

Cystic Fibrosis: Comparison of Two Mucolytic Drugs for Inhalation Treatment (Acetylcysteine and Arginine Hydrochloride)

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ABSTRACT. Clinical, bronchoscopic, spirographic, scintigraphic, and chemical analyses were done in 24 children with cystic fibrosis to assess the mucolytic effects of acetylcysteine inhalations versus L-arginine hydrochloride aerosols. The latter drug is less active than acetylcysteine and should not be used to treat children with cystic fibrosis. *Pediatrics*, 55:000, 1975, CYSTIC FIBROSIS, AEROSOLS, ACETYLCYSTEINE, ARGININE HYDROCHLORIDE.

The choice of drugs for clinical application of aerosol therapy in patients with cystic fibrosis has provoked controversy since its introduction. Many antibiotics, anti-inflammatory, broncholytic, and mucolytic drugs have been used. Enzymatic agents and detergents have been administered in an attempt to liquefy thick secretions. The ideal mucolytic agent should be inexpensive, stable, nonirritating, and effective in liquefying mucus, purulent secretions, and fibrin. Such an agent is not yet available.[1] During the past ten years acetylcysteine has been shown to approach this ideal most closely.[1-13] It has good mucolytic properties and is well tolerated. The only side effects are bronchospastic reactions in some patients, especially those with bronchial asthma.[14] This can be compensated for by administration of isoproterenol. Acetylcysteine is moderately priced, but attacks all rubber and metal equipment, and has the unpleasant odor of hydrosulfuric acid.

Matthews and Doershuk[15] have not been able to document the effect of mucolytic agents objectively. We have shown favorable effects of intermittent inhalation therapy with acetylcysteine in 33 children with cystic fibrosis.[16] Therefore, any new mucolytic drug must be compared with acetylcysteine.

Solomons *et al.*[17] introduced buffered L-arginine into aerosol therapy of cystic fibrosis because of its low toxicity and its ability to break hydrogen bonds, bind metal ions, and act as a detergent. They administered it by inhalation as an ultrasonicated mist to eight patients (ages ranged from 6 to 12 years). They found improvements in vital capacity, thoracic gas volume, specific conductance, and arterial P_{O_2}. No important side effects of this treatment could be detected. Miller reported only that L-arginine inhalations are well tolerated and very effective but did not mention detailed studies.[9] According to Huang, "L-arginine may be a promising agent for intermittent aerosol treatment but must be investigated further in regard to its beneficial as well as adverse effect." We have tried to do so by complex investigations including lung function testing, lung perfusion scintigraphs, and bronchoscopies. Instead of buffered L-arginine base we used buffered L-arginine hydrochloride.

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MATERIAL AND METHODS

Twenty-four patients with cystic fibrosis who were under treatment and supervision in our clinic's outpatient department for more than 18 months received inhalations with arginine hydrochloride. The average age of these children was 6 years and ranged from $2^{6}/_{12}$ to $12^{7}/_{12}$ years. Sixteen patients were boys and eight girls. In all children we performed bronchoscopic examinations before and after the period of treatment with arginine hydrochloride inhalations. Table I shows the results of the bronchographic investigations. In our experience, bronchoscopy and bronchography are good methods to evaluate the course of cystic fibrosis and the effect of therapeutic measures.[3,4,16] For these investigations all children were taken to the clinic before and after the treatment period with arginine hydrochloride. Before beginning arginine hydrochloride inhalations the complex treatment consisted of substitution of pancreatic enzymes, drainage of bronchial secretions, and inhalations of 10% acetylcysteine solutions (Mucosolvin*) with 0.01% isoproterenol (Novodrin†). Aerosols produced at home by using ultrasonic nebulizers (USI 2, USI 3, or USI 4‡) were given to 21 children; in three children two different types of jet nebulizers were employed. The nebulized liquid was 10 to 16 ml per inhalation in ultrasonic nebulizers and 4 ml in jet nebulizers. The daily inhalation time was 10 to 20 minutes in most children.

For the first investigation all children remained in the hospital for a few days. When they were at home the same treatment was continued, but a 5% arginine hydrochloride solution (buffered with sodium hydroxide to a pH of 7.0) was used for the inhalations. The period of treatment with arginine hydrochloride was four to ten (mean, seven) weeks. At the end of this period the children were again studied in the clinic and all investigations were repeated (Table II).

The parents of all patients were informed about the method and intention of our investigations and consented to it.

Statistical evaluation of the mean values and paired comparisons were done by Student's *t*-test.

RESULTS

Inhalations with arginine hydrochloride had to be stopped in five children because of severe deterioration of their general condition and the appearance of an intensive cough. This happened in one child after one week of treatment, in three children after two weeks, and in another child after three weeks. Cough increased continuously during arginine hydrochloride inhalations and disappeared when this drug was replaced by acetylcysteine. No concurrent catarrhal infections could be detected in these five children as a possible cause of the deterioration.

TABLE I

BRONCHOGRAPHIC FINDINGS IN 24 CHILDREN WITH CYSTIC FIBROSIS

Finding	*No.*
Normal bronchial tree	1
Slight bronchitis deformans	6
Moderate bronchitis deformans	2
Severe bronchitis deformans	1
Cylindrical bronchiectasis in 1 or 2 segmental bronchi only	4
Cylindrical bronchiectasis in more than 2 segmental bronchi	8
Saccular bronchiectasis	2

TABLE II

EXAMINATIONS BEFORE AND AFTER TREATMENT WITH ARGININE HYDROCHLORIDE INHALATIONS (24 CHILDREN)

Bronchoscopy—general anesthesia with barbiturates and muscle relaxation with succinylcholine (Friedel's ventilation bronchoscope)
Bacteriological investigation of bronchial secretions
Perfusion scintigraphy with 113^{m} indium-iron hydroxide particles (Picker Magna-Scanner 500)
Lung function and blood gases: (1) Vital capacity (Godart Pulmotest); (2) FEV_1 (Godart Pulmotest); (3) Functional residual capacity (helium dilution technique); (4) Po_2 (Metra measuring chamber with platinum leads); (5) Pco_2 (Meinsberg pH-measuring chain); (6) Po_2 during load (step test for children under 5 by the method of Hettinger and Rodahl and Zimmermann's bicycle ergometer for older children); (7) alveolar arterial oxygen gradient calculated from Vo_2 and Vco_2 minute ventilation (spirolyt and gas meter); (8) Dead space ventilation (calculated physiological dead space to tidal volume ratio; (9) alveolar oxygen partial pressure
Blood chemistry: (1) Calcium; (2) Anorganic phosphate; (3) Creatinine (Jaffé's reaction, analyzing automaton); (4) Urea nitrogen (analyzing automaton); (5) SGPT (ultraviolet test); (6) SGOT (ultraviolet test)
Hematology: (1) Hemoglobin (hemoglobin cyanide method); (2) RBC (electronic counting by the Zählgerät types I and II); (3) Leukocytes (electronic counting by the Zählgerät types I and II); (4) Reticulocytes; (5) Thrombocytes
Urinanalysis: (1) Albuminuria; (2) Glucosuria; (3) Sedimentation analysis

*VEB Berlin-Chemie, Berlin, GDR.
†VEB Chemische Werke Berlin-Grünau, GDR.
‡VEB Transformatoren- und Röntgenwerk Dresden, Werk Hohen Neuendorf, GDR.

TABLE III

RESULTS OF LUNG FUNCTION AND BLOOD GASES TESTING BEFORE AND AFTER ARGININE HYDROCHLORIDE INHALATIONS

Measure	*VC*	*FEV_1/VC%*	*FRC*	*Po_2*	*Pco_2*	*V_D, phys./V_t*	*(A-a) Oxygen Diff.*	*Po_2 During Load*
Normal values	>80%*	>70%	–†	>80 Torr	<44 Torr	<17%	>20 Torr	±7 Torr
No. of patients	8	7	8	18	18	16	16	11
Pathologic values before inhalations	2 1	4	7	6	1	7	13	2
Pathologic values after inhalations	1	4	6	5	0	11	16	1
Mean difference of paired comparisons	0.14±0.0	2.7±4.3	0.13±0.25	0.01±7.6	2.7±4.09	7.57±10.24	1.05±9.36	–
Significance	.47<*P*<.45	.10<*P*<.05	.10<*P*<.05	.49<*P*<.47	.01<*P*<.005	.005<*P*<.0025	.35<*P*<.30	–
No. of paired comparisons with rise	4	1	6	8	1	12	9	6
No. of paired comparisons with fall	4	5	2	8	17	4	7	5
No. of paired comparisons with equality	0	1	0	2	0	0	0	0

*Normal values in relation to height, Dietzsch.[18]

†Normal values in relation to height, Cook and Hamann.[19]

In the remaining 19 children control investigations were done four to ten weeks after the onset of inhalations with arginine hydrochloride. Bronchoscopy revealed a deterioration of the endoscopic picture in most children. Summarizing all endoscopic signs of inflammatory processes (reddening and edematous swelling of the mucous membranes, hypersecretion), the bronchoscopic aspect had deteriorated in 15 children and improved in only 3 children (Fig. 1). Hypersecretion alone had increased in 15 children, diminished in 2 children, and not changed in another 2 children (Fig. 2).

According to scintigraphic examination, the lung perfusion was unchanged in nine children, deteriorated in four children, and improved in three children. The scintigrams of three other children could not be evaluated.

Table III shows the results of lung function testing and blood gas determinations. The decrease of the Pco_2 and the rise of the dead space ventilation are highly significant. This must be considered as deterioration, hyperventilation, and increased distribution disturbances. In most children forced expiratory volume and functional residual capacity had increased at control examination. This means that obstructive ventilation disturbances had increased. Other parameters—Po_2 during rest and load, alveolar-arterial oxygen-gradient—had risen in some patients and fallen in others.

Bacteriologic investigations of the bronchial secretions yielded pathologic organisms before beginning the inhalations with arginine hydrochloride in 17 of 19 patients. The following organisms were found: *Staphylococcus aureus* (14), once in combination with *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* (1), *Diplococcus pneumoniae* (1), and enterococcus (1). Control examinations of these children after arginine hydrochloride inhalations demonstrated staphylococci in 13 children; in the abovementioned three children the other organisms had disappeared.

The parents of our patients registered the days on which the children had coughs. We put the number of these "cough-days" in relation to the number of days during which arginine hydrochloride inhalations were performed and the same number of days before and after this treatment on which the children received acetylcysteine inhalations. The mean values were the following: (1) first acetylcysteine inhalation period, 2.9%; (2) arginine hydrochloride inhalation period, 23.1%;

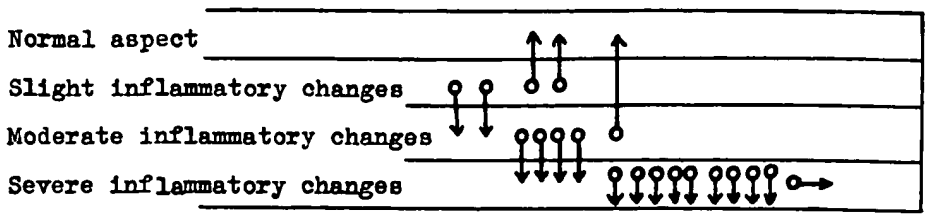

FIG. 1. Bronchoscopic aspect before and after arginine hydrochloride inhalations. *Circles* = before treatment with aerosols; *arrows* = after treatment with aerosols.

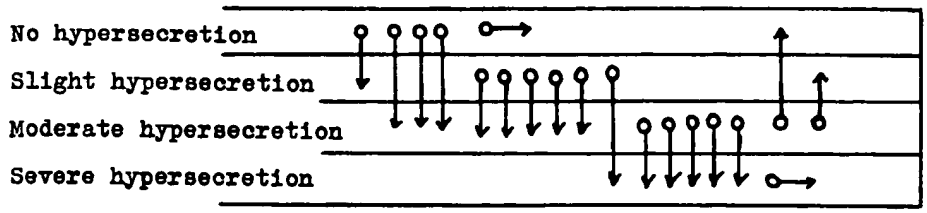

FIG. 2. Bronchial hypersecretion before and after arginine hydrochloride inhalations. *Circles* = before treatment with aerosols; *arrows* = after treatment with aerosols.

and (3) second acetylcysteine inhalation period 9.8%.

FINAL CONCLUSION

Our investigations demonstrated clearly that L-arginine hydrochloride produced few effects. It is less active as a mucolytic agent than acetylcysteine. We cannot recommend the use of L-arginine hydrochloride for this purpose.

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ADULT-TYPE CANCERS IN CHILDHOOD

Important clues to the origins of adult cancers may come from studies of their rare occurrence in childhood. Niitu *et al.* have described their own case of primary lung cancer and nine from the literature in Japanese children under 16 years of age (Am. J. Dis. Child., 127:108, 1974). They found 29 other cases in the literature from the rest of the world. Unfortunately, the histories were not explored, except for their own case, so no new etiologic information was developed. Also, other unpublished cases in Japan might have been found in the Annual of the Pathological Autopsy Cases in Japan. This reference contains a summary of all autopsies performed in that country—about 15,000 annually, of which 400 are children with cancer. Through the use of this resource, 1957 to 1966, five previously unreported cases of pancreatic carcinoma in children were added to seven others in the Japanese literature. The 12 cases constitute one third of the total reported to date throughout the world (Tsukimoto, *et al.*: Pancreatic carcinoma in children in Japan. Cancer, 31:1203, May 1973). When adult-type cancers are seen in children, inquiry into the environmental history may reveal exposures of etiologic interest.

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EXHIBIT 11

D1 13

Anales Españoles de Pediatría
(Spanish Annals on Pediatrics)

INHALED AZTREONAM THERAPY IN PATIENTS WITH CYSTIC FIBROSIS COLONIZED WITH *"Pseudomonas aeruginosa"*

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Tratamiento con aztreonam inhalado en enfermos con fibrosis quística colonizados por "Pseudomonas aeruginosa".

Resumen. Se llevó a cabo un estudio, durante 15 meses, en 19 enfermos con fibrosis quística (FQ) y colonización crónica endobronquial por Pseudomonas aeruginosa (PA), para evaluar la eficacia de aztreonam (antibiótico monobactámico con actividad anti-pscudomona) sobre la densidad de PA. Los pacientes inhalaron una solución salina de 0.5 a 1 g de aztreonam nebulizada dos veces el día por un compresor de alto flujo según pauta discontinua: si la densidad de PA disminuía por debajo de 10 6 unidades formadoras de colonias (UFC/ml), suspendían el tratamiento hasta nueva elevación en el control mensual de cultivo de esputo o antes si presentaban exacerbación clínica. Un enfermo fue excluido por presentar broncoespasmo, y se despreciaron los datos de 2 enfermos por falta de controles regulares. En 15 de los 16 enfermos restantes disminuyó la densidad de PA por debajo de 10^6 UFC/ml. Dicha disminución y su mantenimiento ulterior no guardó relación con un número determinado de ciclos de tratamiento previo. Aparecieron cepas de PA resistentes a aztreonam de forma intermitente. En el 85,7% de los enfermos se observó mejoría clínica y radiológica. En el 80% de los 10 enfermos con espirometrías valorables mejoraron los parámetros capacidad vital forzada (CVF) y volumen espiratorio en un minuto (FEV 1). En 2/16 se elevaron las aminotransferasas. Concluimos quo el aztreonam inhalado reduce la densidad de PA, pero siguiendo una pauta discontinua no se puede mantener una densidad mínima constante.

Palabras clave: Tratamiento fibrosis quística. "Pseudomona aeruginosa". Aztreonam inhalado.

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INHALED AZTREONAM THERAPY IN PATIENTS WITH CYSTIC FIBROSIS COLONIZED WITH "PSEUDOMONAS AERUGINOSA"

Abstract. An 18-month study with nineteen patients with cystic fibrosis (CF) and endobronchial colonization by Pseudomonas aeruginosa (PA) was carried out in order to assess efficacy of aztreonam, a monobactam antibiotic with anti-pseudomonal activity, on P. aeruginosa density. Twice a day the patients inhaled 0.5 to 1 gram of aztreonam saline solution that was nebulized via a high flow compressor according to an intermittent treatment protocol. If the PA density decreased below 10^6 CFU/ml, the treatment was stopped until the following monthly sputum cultures showed a new increase in density or before if pulmonary exacerbation was present. One patient was excluded from the study because he had a hypersensitivity reactivation, and data from two other patients were ignored due to the lack of regular controls. PA density decreased below 10^6 CFU/ml in 15 of 16 remaining patients. This reduction and subsequent maintenance thereof were not related to a fixed number of previous treatment cycles. Strains of PA resistant to aztreonam occurred intermittently. Clinical and radiological improvements were seen in 85.7% of the patients. Forced vital capacity (FVC) and forced expiratory volume (FEV 1) improved in 80% of the 10 patients with evaluable pulmonary function. Elevated aminotransferases were observed in 2 out of the 16 patients. We conclude that nebulized aztreonam reduced PA density, but a constant minimum density cannot be maintained following an intermittent treatment protocol.

Key words: Cystic fibrosis treatment, Nebulized aztreonam, Pseudomonas aeruginosa.

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INTRODUCTION

Cystic Fibrosis (CF) is an autosomal recessive disease that affects 1out of every 2500 live births among the white population. The survival rate has improved, and it is estimated that the median life of a child born with CF (whom receives appropriate care) is 40 years old. The latter, not taking into account future therapeutic advances (1).

Chronic infection by *pseudomonas aeruginosa* is the main cause of progressive lung damage and death in 99% of the cases. Furthermore, parenteral quimioprofilaxis requires high dosing due to the poor concentration attained in the respiratory tissue and secretions where the infection localizes (2,3). Failure to eradicate Pseudomonas and high toxicity has prompted the search for other administration routes and new drugs. Oral administration of ciprofloxacin quinolone is limited for its possible toxicity and appearance of resistant strains (4). Administration of the drug via bronchial tree, by depositing the drug directly in the airways (5), achieves technical advantages of higher local concentration of the drug, and minimal systemic absorption (6).

The goal of this study was to evaluate the efficacy of aztreonam (a monobactam antibiotic with anti-pseudomonal activity) to reduce the bacterial density of *P. aeruginosa*, administered by inhalation in intermittent fashion, in patients with CF and chronic lung disease. Also to evaluate the clinical correlation of such efficiency, do radiology and pulmonary function studies, and to study possible secondary effects of the drug, and appearance of resistant strains.

Materials and Methods

Nineteen patients ranging in ages from 4 to 20 years old were selected for the study. Such patients controlled at the CF Unit of the University Hospital "Virgen del Rocío" in Seville, were diagnosed with CF and presented with chronic lung disease, pancreatic insufficiency and elevated sweat chloride test. All of them were being treated routinely with physical therapy, hyper caloric diet, pancreatic enzymes, and vitamins. They were chronically colonized with *P. aeruginosa* for over a year, with the exception of two patients: one less than a year, and other less than 6 months. None of them had received any treatment against Pseudomonas for at least a month prior to the study. According to grading: Clinical by Showman (7), radiologic by Chrispin-Norman and Brasfield (8,9), and respiratory function tests, the clinical grade of the disease was considered as moderate in 8 patients, mild in 2 patients, moderate-severe in 4, and severe in 1.

Table I Clinical and radiologic characteristics of 16/19, and pulmonary function of 10/16 patients with cystic fibrosis

Parameter (mean)	*Treatment initiation*	*End therapeutic period*	*p*
Height and weight [a]	91	95	<0.05
Clinical grading[b]	5	3	<0.01
RX grading[c]	12	9	<0.05
RX grading[d]	9	7	<0.05
FVC[a]	70	72	NS
FEV[a]	56	60	NS

[a] % of ideal for his (her) size
[b] Optimal value = 0; Severe = 10.
[c] Chrispin-Norman optimal value = 0 (8).
[d] Brasfield optimal value = 0 (9).

A nebulized solution of aztreonam (Azactam®) 500 mg (< than 5 years of age), or 1 gr (older than 5 years of age) was administered through a CR60 System 22 unit (Media- Aid limited) twice per day, after a respiratory physical therapy session. Prior to physical therapy, the patients inhaled 3 cc of normal saline solution alone in aerosol, or mixed with salbutamol, or ipratroprium bromide and fenoterol bromohidrate, if shown effective by spirometry. The patients were taught to make deep and slow inhalations, retaining the inhaled solution prior to expiring normally. An intermittent treatment was selected: one treatment cycle lasted 21 days, and after this, the patients rested during a week followed by a morning collection of sputum and continuation of treatment, until sputum result was available. If the colony count for *P. aeruginosa* was < 10^6 CFU/ml, the treatment was suspended. A new cycle was initiated if the next control of sputum culture exceeded a 10^6 CFU/ml colony count, or before, if the clinical symptoms were exacerbated (more frequent cough, increase of sputum volume, and fever). In two patients, the treatment was switched to a continuous protocol after approximately 9

months in order to avoid an improper completion of the intermittent treatment. Oral treatment was prescribed in the presence of *Staphylococcus aureus* confirmed by culture and sensitivity testing.

Clinical and sputum controls were done every month during a period of 18 months. The clinical grading system that was used took into consideration the following parameters: cough frequency, sputum volume, fever, tachypnea, use of accessory muscles, cyanosis (0-3 points) and increase/decrease of weight and appetite (0/1p).

Every six months analytical controls of CBC, liver enzymes, glucose, urea, creatinine, urine testing and capillary blood gases were done. In addition, chest X rays, height/weight (H/W) relation (10) and respiratory function tests (if patient collaborated) were also done every six months. The H/W ratio was expressed as a percentage of the ideal weight. The same radiologist who had no prior patient information, by means of the usual grading criteria, evaluated all the X rays.

Bacteriologic methodology: All the sputum samples were analyzed maximum 30-60 minutes after collection time. First, they were homogenized with N-acetylcysteine (Fluimicil®) at variable proportions according to the sputum consistency. With the purified homogenate, serial dilutions were made in PBS (1/10,1/100,1/1000 and 1/10000) and 10 µl of each were inoculated in blood agar, enriched chocolate agar, blood agar with penicillin, mannitol agar, Levine EMB and Saboureaud agar with dextrose and gentamicin. After incubating at 37°C during 24 hours, colony count and identification of the different pathogens was done with API Number E for the Pseudomonas strains.

The microbiologic criteria used to determine infection was the following: *P. aeruginosa* CFU/ml > 10^6, *Staphylococcus aureus* CFU/ml > 10^5, and *Haemophilus influenza* CFU/ml > 10^5. Statistical analysis was done with the Sigma Plus program.

Results

Three of the nineteen patients that participated in the study were excluded: One for developing bronchospasm after the first and second doses of aztreonam, and the other two for failure of performing the bacteriologic controls on time.

Eleven of the 16 patients that were evaluated presented with evidence of clinical improvement: less cough and sputum volume (< 25 ml/day), and increased appetite and weight. Their initial clinical grading decreased by two or more points. Five of the sixteen patients maintained low-moderate symptoms throughout the study.

Eight of the sixteen patients had exacerbations of moderate intensity during the periods in between treatments, which corresponded with a new increase in the colony count of *P. aeruginosa*. Only one female patient (10 years and 5 months) required to be hospitalized for severe respiratory exacerbation that can be attributed to poor compliance. For this reason, it was decided to switch her treatment, and that of another patient severely affected, to a continuous protocol and a more stringent control. In this way, clinical improvement was achieved again, but as the first patient reverted to his original height weight percentile, the second one recuperated his weight but not his height/weight ratio.

At the end of the study, the percentage of ideal weight for the size was increased by 3-10% in 9/16 patients. Four patients maintained the same percentage throughout the study (two with perfect value, and the other two with an acceptable value) and three had a decreased percentage equal to 4%.

The radiologic image values improved in 10/16 patients by decrease or disappearance of infiltrates and large shadows (the X ray grading decreased by two or more points), in 4/16 they remained the same, and in 2/16 they worsened (see Table I).

Table II Variations of the initial colonization with *P. aeruginosa* > 10^6 CFU/ml in the sputum culture throughout the study, and other changes.

Decrease < 10^6 CFU/ml	10/16
Change to negative	5/16
No variation	1/16
Resistance	10/16
Isolation of *S. aureus*	11/16
Isolation of *H. influenza*	1/16
Isolation of *A. fumigatus* or *C. albicans*	4/16
Isolation of *P. cepacia*	1/16

In 90% of the cases the clinical grading matched the X rays and the H/W percentile. An improvement in all of these parameters

(including the improvement concept of maintenance without change at a good level) was observed in 87.5% of the cases.

Bacteriology Table II: In fifteen out of sixteen (15/16) patients the number of colonies of *P. aeruginosa* per milliliter decreased below 10^6 CFU's. In one out of sixteen patients (1/16) the initial count above 10^6 CFU/ml remained unaltered. A decrease in the colony count and maintenance at that level had no correlation with the number of previous treatment cycles. The density of P.A. would increase again when the treatment was suspended after a variable cycle of treatment recess, and was associated with clinical exacerbation. The resistance of *P. aeruginosa* to aztreonam appeared in 10/16 patients, and in no more than 4 intermittent times in random fashion. The resistant colonies disappeared after substituting aztreonam for tobramycin. Oral antibiotic therapy against staphylococcus (according to susceptibility testing) was associated in 11/16 cases in which *S. aureus* was detected, and it had a 50% coincidence with a decrease in the colony count of *P. aeruginosa*. The isolation of *A. fumigatus* was not correlated with clinical symptoms of allergic bronchopulmonary aspergillosis, and as with the isolation of *C. albicans*, it only occurred in low numbers and in isolated cultures. *Pseudomonas cepacia* was only isolated in one patient.

The results of the pulmonary function tests only allowed for evaluation in 10/16 patients: In 5/10 the FVC and FEV1 parameters improved, in 3/10 the same normal levels were maintained, and in 2/10 they worsened, and this was manifested with clinical worsening of the symptoms and the X rays. The analysis of capillary blood gases had minimal variation throughout the study.

A direct positive correlation was encountered between appropriate completion of treatment guidelines, and clinical and bacteriological improvement.

No alterations in any of the blood lineages, PT or BUN and creatinine levels were encountered that could suggest toxicity of aztreonam produced by its absorption from the bronchial surface. One patient had elevated values, which doubled the baseline levels of SGOT and SGPT, and another had values that fluctuated throughout the study. However, both patients had initial baseline levels equal to 1.3 times the normal upper limit for aminotransferases.

Discussion

Antibiotic therapy in aerosol for the treatment of CF has been used since the 40's. However, its use is still controversial due to discrepancies in the results encountered by the studies (6,11,12).

There are a large number of studies that favor the use of nebulized antibiotics on the basis of achieving a reduced rate of disease exacerbations and hospitalizations, which are accomplished with this therapeutic modality. Additionally, they suggest improvement in the quality of life of the CF patient. In these studies, when periods with antibiotic treatment are compared with periods with placebo treatment, it has been observed that the pulmonary function is improved in the treatment periods (13,14,15,16).

Antibiotic therapy in aerosol does not accomplish complete eradication of *P. aeruginosa* from the respiratory pathways of a patient with CF. However, it reduces the antigen load derived from this organism, and thus, a continuous reduction decreases the formation of immune complexes and hypersensitivity reactions type III, which are believed to contribute largely to the lung tissue damage in this type of patients (17).

Only a minimal number of collateral effects have been described (26), and no hypersensitivity reactions, resistance to antibiotics used, nor candidiasis have been encountered (18).

In order for an antibiotic that is applied in aerosol form to be effective, it is required that a sufficient amount of it reaches the site of infection, and that it remains at this site long enough for it to exert its bactericidal activity (5,19). In order to attain this objectives there are a number of requirements: The diameter of the aerosol particles must be between 1-5 microns, the technique to be followed in the patient will consist of long and deep inhalations holding the breath to the end of the inhalation, and the respiratory pathways should not be obstructed (20).

The amount of particles deposited in peripheral airways is inversely proportional to the patient's FEV1 (21). But in the patient with severe CF disease, there are several factors that interfere

with proper distribution in the respiratory tree (12).

Despite the fact that the microbial flora of the lower airways is more complex than that of the sputum (23), the positive secretion cultures of the upper and lower airways in the patients with CF match (22).

Aztreonam is a monobactam antibiotic with anti-pseudomonal activity. The spectrum of activity of this drug is limited to Gram-negative aerobic bacteria, in a similar fashion to that of amynoglycosides, but with very low toxicity (24).

The results of this study proved that the treatment with aztreonam was effective because the goal of reducing the colony number of *Pseudomonas aeruginosa (PA)* below the limit previously set (10^6 CFU/ml) was attained. However, you cannot set a precise number of treatment cycles required to decrease the density of *P.A.* The latter may be explained because the patients did not do the physical and aerosol therapy with a good technique on a regular basis.

We base this comment from our study observation of a direct correlation between clinical and bacteriologic improvement with proper treatment technique. In addition, because of the way the respiratory tree was affected in a group of patients, which was different from another group, and in one isolated patient; as it related to the different treatment cycles. The difference consisted in larger or smaller areas of difficult drainage.

The intermittent treatment protocol offers some advantages to the patient: For example, it allows him to enjoy more free time and to forgo during one or a couple of months an aggressive antibiotic treatment that affects the respiratory tree (25). However, if the ultimate goal is to attain a constant *P.A.* density, and a continuous reduction of the antigenic load derived from the bacteria, a continuous treatment protocol is preferable, even if it requires the aid of regular anti-pseudomonal oral or I.V antibiotic therapy (17).

Aztreonam resistance does not seem to be a major problem, because it was always very short, as the resistant *PA* colonies disappeared soon after treatment with tobramycin.

Among the secondary effects of treatment only one case of bronchial hypersensitivity was observed. In addition, two patients presented with elevated SGPT and SGOT enzymes, and there was doubt as to whether this was related to the treatment or not.

Eighty five point seven percent (85.7%) of the patients treated with an intermittent protocol of aztreonam in aerosol experienced decreased symptoms, no changes in their chronic X ray patterns, but a disappearance of patchy infiltrates and atelectatatic images, and improved or maintained normal values in their pulmonary functions. It is presumed that the progressive pulmonary deterioration was delayed for a while due to less *P.A.* infections. The subsequent appetite increase brought about a larger and progressive weight gain. And all of the above was translated into a clear improvement in the quality of life of these patients.

Conclusion

Our study demonstrates the efficiency of an intermittent treatment with aztreonam in aerosol, an anti-pseudomonal agent, as it relates to decreasing the density of *Pseudomonas aeruginosa* in patients with cystic fibrosis, which are chronically colonized by this microorganism. The advantages of using an intermittent treatment protocol of aztreonam by inhalation do not overcome the inconvenience of not being able to maintain consistently a minimally reduced number of *P. aeruginosa*.